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ERRATA

Page 32, Table XX, "*Trifolium repens*" should read "*Trifolium pratense.*"

Page 52, Table XXX, "*Linum usatissimum*" should read "*Linum usitatissimum.*"

Page 52, Table XXX, "*Cucumbis sativa*" should read "*Cucumis sativus.*"

Page 53, Table XXX, "*Boutelona gracilis*" should read "*Bouteloua gracilis.*"

Page 53, Table XXXI, "*Cucumis sativa*" should read "*Cucumis sativus.*"

Page 56, Table XXXIII, column head, "Ratio, 1913 to 1912," should read "Ratio, 1912 to 1913."

Page 56, line 4 from bottom, "75±2" should read "75±1."

Page 97, line 26, "*Salix nutallii*" should read "*Salix nuttallii.*"

Page 106, Table II, "*Sutanion velutinum*" should read "*Sitanion velutinum.*"

Page 165, Table III, insert line after "MgO" to read "Fe_2O_3....0.07 0.10 0.09 0.10."

Page 165, Table IV, "Fl_2O_3" should read "Fe_2O_3."

Page 165, Table IV, "Total..... 99.94 ..... 98.36 ....." should read "Total..... 99.94 99.54 100.36 100.00."

Page 166, Table V, "Total..... 104.45 104.82 101.41 101.99" should read "Total ..... 99.73 100.04 99.25 99.81."

Page 256, line 10, "($C_1=t-t_m$; $C_2=C_1^2-(C_1^2)$; $_m$ $N=D_t-(D_t)_m$.)" should read "$C_1=t-t_m$; $C_2=C_1^2-(C_1^2)_m$; $N=D_t-(D_t)_m$."

Page 256, line 11, "($\Sigma C_1^2\alpha+\Sigma C_{12}\beta C=\Sigma C_1 N$.)" should read "$\Sigma C_1^2\alpha+\Sigma C_1C_2\beta=\Sigma C_1N$."

Page 278, lines 37–38, "(fig. 2)" should read "(fig. 3)."

Page 279, line 19, "as" should read "at."

Page 279, legend for figure 2, "Side views of basal portions, etc.," should read "Fig. 2.—Detail drawings of ventral view of seeds of *Agropyron* spp.: *A*, *Agropyron repens; B*, *A. smithii; C*, *A. tenerum.* × 9."

Page 280, legend for figure 3, "Detail drawings of ventral view, etc.," should read "Fig. 3.—Side views of basal portions of seeds of *Agropyron* spp., showing the relative projection of the rachilla: *A*, *Agropyron repens; B*, *A. smithii; C*, *A. tenerum.* × 9."

Page 280, legend for figure 4, "Edge of rachilla in *Agropyron* spp., etc.," should read "Fig. 4.—Edge of palea in *Agropyron* spp., showing space and comparative size of bristles: *A*, *Agropyron repens; B*, *A. smithii; C*, *A. tenerum.* × 9."

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RELATIVE WATER REQUIREMENT OF PLANTS

By LYMAN J. BRIGGS, *Biophysicist in Charge, Biophysical Investigations*, and H. L. SHANTZ, *Plant Physiologist, Alkali and Drought Resistant Plant Investigations, Bureau of Plant Industry*

INTRODUCTION

The marked differences in the quantity of water required by different species of plants for the production of a given weight of dry matter when grown under the same environmental conditions is a matter of scientific interest and of great economic importance in regions of limited water supply. The measurements which have heretofore been made have for the most part been limited to a few species and have been carried out under such varied environmental conditions that comparison is difficult. The writers have therefore undertaken the measurement of the water requirement of representative species and varieties of the principal crop plants, grown at the same place and under as nearly uniform conditions as to time as the temperature requirement and life history of the different crops will permit. The first series of measurements were made at Akron, Colo., in 1911 (Briggs and Shantz, 1913a)[1]. These measurements were extended in 1912 and 1913 to include many species whose water requirement had never before been determined. The later measurements form the subject of the present paper. The writers desire to express their obligation to Messrs. R. D. Rands, A. McG. Peter, H. Martin, F. A. Cajori, N. Peter, and G. Crawford for efficient and painstaking assistance in connection with these experiments.

EXPERIMENTAL CONDITIONS

The experimental procedure in 1912 and 1913 was similar to that in the earlier experiments. The plants were grown to maturity in large galvanized-iron pots holding about 115 kg. of soil. Each pot was provided with a tight-fitting cover having openings for the stems of the plants, the annular space between the stem of the plant and the cover being sealed with wax. The loss of water was thus confined almost entirely to that taking place through the leaves, and the entrance of rainfall was almost wholly excluded. The wax which has been found to be the most

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 62-63.

satisfactory for sealing the openings about the stems consists of a mixture of four parts of unrefined beeswax with one part of tallow.

Six pots of plants of each variety [1] were used, and the water requirement of each pot was determined independently, in order to provide a basis for the calculation of the probable error of the mean. In making this calculation, Peter's abridged method, based upon the sum of the departures, has been employed.[2]

The term "water requirement," when employed in the following pages without further restriction, indicates the ratio of the weight of water absorbed by a plant during its growth to the weight of the dry matter produced, exclusive of the roots (Briggs and Shantz, 1913a, p. 7). The plants were dried to constant weight in a steam-heated oven, maintained at approximately 110° C. When the plants produced grain, the water requirement based upon the weight of the dry grain is also given. The percentage of grain produced by the plants grown in the pots usually compared favorably with the field performance. Unless a normal percentage of grain is produced, the water-requirement ratio based on grain production should not be applied to crops grown under field conditions. In a few instances the water requirement based upon the weight of roots or tubers has also been determined.

SCREENED INCLOSURE

To protect the plants from birds and severe hail and wind storms, it was found necessary to conduct the experiments in a screened inclosure. The inclosure used in 1911 consisted of a wooden framework covered with wire netting of ¼-inch mesh. This framework shaded the plants somewhat, being made sufficiently rigid to support a track above each row of cans, from which the cans were suspended during weighing. To reduce the shading effect, a new inclosure was provided in 1912, the framework of which was made of 1-inch galvanized-iron pipe with pipe posts 9 feet high at intervals of 8 feet. The framework to a height of 3 feet was covered with a wooden wall which came slightly above the top of the pots. The remainder was covered with No. 21 galvanized-wire netting of ⅜-inch mesh. General views of the inclosure are shown in Plate I.

Although the new inclosure reduced the shading effect, pyrheliometric and total radiation measurements made inside and outside the inclosure still showed a measureable reduction in the radiation due to the shade of the screen. Measurements made with an Abbot silver-disk pyrheliometer (Abbot, 1911) showed that the intensity of the direct radiation

[1] The recorded strains used in these measurements were obtained from the following offices of the Bureau of Plant Industry: Foreign Seed and Plant Introduction (S. P. I.); Cereal Investigations (C. I.); Alkali and Drought Resistant Plant Investigations (A. D. I.).

[2] The formula used was $R_m = 0.845 \frac{\Sigma d}{n\sqrt{n-1}}$, where R_m = the probable error of the mean, Σd = the sum of the departures, and n = number of determinations.

A probable error based upon six determinations does not necessarily represent strictly the actual frequency diagram, and this must be borne in mind in the consideration of probable errors. For a discussion of the probable error when the number of observations is small, see "Student" (1908).

from the sun was reduced about 20 per cent by the inclosure at midday in midsummer, while total radiation measurements made with a differential telethermograph (Briggs, 1913) gave approximately the same reduction. Simultaneous measurements of the water requirement of wheat, alfalfa, and cocklebur grown inside and outside the inclosure in 1913 showed that the inclosure reduced the water requirement about 22 per cent. The water-requirement measurements must therefore be considered relative rather than absolute. In this connection it should be recalled that plants growing under field conditions are also mutually shaded and otherwise protected to some extent. The writers' measurements in 1913 show that wheat grown in pots sunk in trenches and surrounded by a field of grain has a water requirement 10 per cent above wheat grown in the inclosure and 10 per cent below wheat grown outside the inclosure in a freely exposed wind-swept position. (See Table I.) The stand of wheat about the trench was below normal, owing to the disturbance of the plants in trenching and in caring for the pots. The potted plants in the trenches were consequently more exposed than if growing normally in a field of grain. The water requirement of the potted plants in the trench is therefore somewhat above that of plants normally protected. From this comparison it appears that the inclosure measurements, at least in the case of wheat, are less than 10 per cent below the water requirement of plants exposed under field conditions.

TABLE I.—*Effect of the screened inclosure on the water requirement of wheat at Akron, Colo., in 1913*

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1913.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Kubanka, C. I. 1440 (*Triticum durum*), check series, May 22 to Aug. 13.	7	162.2	56.5	103.8	35	1,837	640
	8	167.4	63.9	105.1	38	1,645	628
	9	143.4	49.0	91.2	35	1,861	636
	10	151.6	51.4	93.1	34	1,810	614
	11	148.9	56.5	89.5	38	1,584	601
	12	159.3	50.3	102.2	32	2,032	642
Mean						1,795±43	627±5
Kubanka, in field, May 22 to Aug. 13.	1	142.5	46.6	81.4	33	1,745	571
	2	151.1	43.9	80.8	29	1,840	534
	3	153.4	48.9	82.9	32	1,694	540
	4	156.0	42.8	88.4	27	2,063	566
	5	143.9	48.2	83.3	33	1,726	579
	6	167.8	44.9	97.3	27	2,167	580
Mean						1,873±61	562±6
Kubanka, in shelter, May 23 to Aug. 13.	73	294.8	106.5	150.3	36	1,410	510
	74	273.4	101.4	135.9	37	1,340	497
	75	257.0	97.1	122.4	38	1,261	476
	76	304.2	116.2	156.0	32	1,342	513
	77	253.3	93.8	121.3	37	1,293	479
	78	299.6	116.8	150.2	39	1,286	502
Mean						1,322±16	496±5

WEIGHING AND WATERING

The discarding of the overhead track necessitated the construction of a movable support for weighing the cans. The weighing support used is shown in Plate II, figure 2. It was constructed of 1-inch galvanized-iron pipe and consisted of a crossbar which spanned the row and which was supported at each end by two bent posts. These posts were fitted with floor plates secured to two wooden skids, which slid along the ground on either side of the row of cans. In the earlier weighings the pots were suspended from a rope running through pulleys to a small windlass located on one of the posts of the support (Pl. II, fig. 2). The windlass was later located directly beneath the crossbar and was operated through a chain-and-sprocket drive.

Each pot was provided with bale ears by which it could be suspended directly from the balance by chains. When the plants were not sufficiently high to come in contact with the weighing apparatus, pots could be weighed at the rate of two a minute if two men handled the support and a third recorded the weight. When 300 pots or more are to be weighed three times a week, as was the case at Akron, rapidity in weighing becomes important.

The initial and final weighings have been made with an accuracy of one-fifth of a kilogram, either with a platform balance or a sensitive spring balance calibrated and corrected for temperature. Intermediate weighings have been made throughout with a spring balance calibrated by means of a sealed check pot weighing 130 kg.

The water in all cases has been added from calibrated 2-liter flasks (Briggs and Shantz, 1913a, p. 11). The neck of each flask is cut so as to deliver 2 liters of water when brimful. The flasks are filled by submersion. In some of the later work a tank with a framework arranged for keeping a number of flasks submerged has been used. No time is thus lost in filling flasks or in adjusting the contents to a fiducial mark.

SOIL FERTILIZER

Surface soil from the experiment farm was used for filling the pots. Since it is well known that the water requirement is increased by a deficiency in the plant food supply (Briggs and Shantz, 1913b, pp. 31–56), the same quantity of a complete soluble fertilizer was added to each pot at each station at intervals during the growth of the crop. The fertilizer in 1912 was applied at the rate of 50 p. p. m. of PO_4, 100 p. p. m. of NO_3, and 65 p. p. m. of K, all based on an assumed dry soil mass of 100 kg. per pot.[1] The phosphoric acid was applied as sodium phosphate; the nitrogen and potash as potassium nitrate. This amount of fertilizer was divided into four equal portions and applied at intervals during the active growth of the crops, the first application being made soon after

[1] Approximately one-half of this quantity was used in 1913 and was applied as in 1912.

the plants had become well established. In practice it was found convenient to make up a large quantity of the fertilizer solution of such concentration that 2 liters contained one-fourth of the total quantity required for one pot. The addition of this quantity to each pot was followed immediately by 2 liters of water.

To test the influence of the fertilizer, one standard set of six pots of Kubanka wheat was grown without fertilizer at Akron, for comparison with the fertilized sets. The detailed results are given in Table II. The water requirement of the unfertilized set was 4 ± 2 per cent below that of the fertilized set when based on the production of dry matter, and 1 ± 4 per cent above, when based on grain production. The results therefore indicate that the additional plant food was not needed at this station, the water requirement of the two sets agreeing (within the errors of the experiment) whether based on the production of dry matter or on the production of grain. In 1911 the water requirement of the unfertilized set [1] at Akron was 6 ± 3 per cent above the fertilized set when compared on the basis of dry matter, although the ratios based on grain production were the same.

TABLE II.—*Effect of fertilizer on the water requirement of wheat at Akron, Colo., in 1912*

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Kubanka, C. I. 1440 (*Triticum durum*), May 9 to Sept. 3, fertilized	1	270. 0	108. 2	95. 1	40	879	352
	2	252. 7	88. 3	97. 1	35	1, 099	384
	3	279. 4	98. 1	109. 9	32	1, 120	393
	4	288. 8	99. 7	118. 6	35	1, 190	411
	5	291. 8	99. 5	122. 0	34	1, 226	418
	6	261. 7	92. 2	106. 2	35	1, 152	406
Mean						1, 111 ± 37	394 ± 7
Kubanka, C. I. 1440, May 9 to Aug. 21, unfertilized	7	291. 8	102. 8	108. 6	35	1, 056	372
	8	272. 3	100. 6	107. 4	37	1, 067	394
	9	290. 3	101. 1	108. 8	35	1, 076	375
	10	274. 8	79. 5	109. 4	29	1, 375	398
	11	300. 2	105. 7	114. 1	35	1, 080	380
	12	263. 2	87. 6	88. 4	33	1, 090	336
Mean						1, 124 ± 32	376 ± 6

CLIMATIC FACTORS

The instrumental equipment for the measurement of climatic factors included maximum and minimum thermometers and an air thermograph exposed in a standard shelter 4 feet above the ground surface, an anemometer, a psychrometer, a rain gauge, and an evaporation tank.

[1] Based on pots 1 to 6, unfertilized, which had the same exposure as pots 7 to 12, fertilized.

The sunshine, the wind velocity, the combined sun and sky radiation, the wet bulb depression, and the evaporation were automatically recorded. The results of some of these measurements, combined in 5-day periods for the sake of brevity, though not without sacrifice, are given in Tables III and IV. The discussion of the influence of climate on water requirement has purposely been restricted, since such correlations as may exist can best be determined when discussed in connection with the results from other stations established for this purpose.

TABLE III.—*Summary of climatic conditions at Akron, Colo., in 1912*

Month.	Days (inclusive).	Air temperature (°F.).					Precipitation.	Evaporation.	Wind velocity per hour.
		Average of—			Maximum.	Minimum.			
		Means.	Maximums.	Minimums.					
1912.							*Inches.*	*Inches.*	*Miles.*
April	1 to 5	48	62	33	73	29	.....	0.82	7.7
	6 to 10	44	60	29	69	23	0.26	.71	8.5
	11 to 15	44	56	30	68	26	.51	.71	14.7
	16 to 20	40	49	29	54	26	.36	.47	9.5
	21 to 25	46	57	32	70	27	.03	.78	9.6
	26 to 30	49	62	35	69	32	1.33	1.09	9.5
May	1 to 5	50	67	35	76	28	.35	.98	10.4
	6 to 10	53	66	41	75	34	.40	.90	8.3
	11 to 15	44	53	36	65	32	.97	.60	8.6
	16 to 20	61	77	47	83	41	Tr.	.99	6.9
	21 to 25	64	78	47	84	42	Tr.	1.32	7.1
	26 to 31	60	77	44	92	35	1.14	2.31	9.3
June	1 to 5	64	78	49	83	45	.....	1.32	7.8
	6 to 10	59	69	49	78	47	.41	.74	8.1
	11 to 15	60	72	49	84	44	1.03	.84	4.5
	16 to 20	55	65	41	78	37	1.51	1.00	4.3
	21 to 25	66	80	50	86	43	.....	1.21	5.5
	26 to 30	72	88	55	89	50	.44	1.64	6.1
July	1 to 5	66	81	49	87	46	.03	1.12	5.1
	6 to 10	73	89	55	96	51	.34	1.58	6.1
	11 to 15	70	86	54	92	51	.01	1.36	5.6
	16 to 20	69	82	55	90	50	.72	1.06	6.1
	21 to 25	73	88	59	94	55	1.63	1.38	5.5
	26 to 31	69	80	57	85	53	.85	1.12	4.1
August	1 to 5	68	79	58	84	54	.16	1.04	7.1
	6 to 10	65	79	50	85	48	.32	1.05	3.8
	11 to 15	69	83	54	89	50	.38	1.15	5.2
	16 to 20	68	82	53	86	51	.56	1.04	3.0
	21 to 25	72	89	56	95	52	.....	1.29	4.0
	26 to 31	72	88	56	96	53	.16	1.48	5.1
September	1 to 5	70	87	54	90	47	.....	1.39	6.4
	6 to 10	65	80	51	91	47	.43	1.08	7.1
	11 to 15	51	61	38	77	32	1.26	.61	5.6
	16 to 20	51	66	37	81	31	.....	.62	6.3
	21 to 25	46	59	34	71	22	.02	.54	6.3
	26 to 30	43	56	32	70	26	.17	.41	4.5

TABLE IV.—*Summary of climatic conditions at Akron, Colo., in 1913*

Month.	Days (inclusive).	Air temperature (°F.).					Precipitation.	Evaporation.	Wind velocity per hour.
		Average of—			Maximum.	Minimum.			
		Means.	Maximums.	Minimums.					
1913.							*Inches.*	*Inches.*	*Miles.*
April	1 to 5	49	66	31	77	21	0.02	0.82	7.4
	6 to 10	33	46	24	73	10	.94	.78	12.4
	11 to 15	45	64	29	77	18	.....	.31	3.6
	16 to 20	53	69	40	74	34	.87	.76	7.2
	21 to 25	44	55	34	68	27	.36	.51	11.0
	26 to 30	60	78	40	84	37	.....	1.15	7.1
May	1 to 5	47	58	35	69	31	Tr.	.72	6.7
	6 to 10	54	68	43	81	41	.82	.84	8.9
	11 to 15	55	69	43	80	33	.54	.86	7.4
	16 to 20	55	70	42	80	38	Tr.	.93	7.6
	21 to 25	62	77	46	84	39	.02	.99	5.7
	26 to 31	69	86	51	91	48	.06	1.50	5.6
June	1 to 5	65	81	51	87	48	.26	1.15	6.2
	6 to 10	57	67	45	77	37	Tr.	1.07	10.3
	11 to 15	66	81	51	91	42	.16	1.17	9.0
	16 to 20	71	88	54	93	52	Tr.	1.27	6.6
	21 to 25	69	86	54	89	52	.51	1.33	5.8
	26 to 30	74	91	60	97	49	.42	2.19	10.3
July	1 to 5	75	92	56	100	53	.....	1.71	7.0
	6 to 10	79	96	60	101	56	.....	1.88	6.6
	11 to 15	74	92	56	103	46	.02	1.78	6.8
	16 to 20	68	83	56	93	53	1.12	1.27	4.8
	21 to 25	66	78	56	87	53	.61	1.01	6.8
	26 to 31	67	87	49	93	43	.10	1.61	5.0
August	1 to 5	78	95	61	98	57	.....	1.75	5.8
	6 to 10	74	90	59	97	54	.05	1.54	6.2
	11 to 15	73	90	59	93	56	.81	1.23	4.9
	16 to 20	76	93	61	95	56	.24	1.38	5.0
	21 to 25	73	90	57	97	53	.....	1.58	5.9
	26 to 31	75	91	59	98	54	.04	1.83	5.9
September	1 to 5	73	90	56	92	54	.17	1.37	4.8
	6 to 10	68	85	53	92	48	.45	1.27	6.7
	11 to 15	62	79	45	86	40	.10	1.30	7.6
	16 to 20	53	68	39	87	29	Tr.	.98	9.2
	21 to 25	45	59	32	76	27	.39	.61	5.6
	26 to 30	50	60	41	70	37	.97	.51	4.7

The months of June, July, August, and September, 1913, were all warmer than in 1912, the average difference in the monthly means being 4° F. In only 2 of the 24 five-day periods into which these months are divided did the mean maximum temperature in 1912 exceed that of 1913. The character of the two seasons is best reflected, however, in the evaporation graphs shown in figure 1. The evaporation for the two years was not essentially different up to the 1st of June. From this time on the evaporation in 1912 averaged much lower than in 1913.

The marked response of the plants to the different seasons is shown in the reduced water requirement in 1912. (See Tables XXXII, pp. 36–38, and XXXIII, p. 39.)

WATER REQUIREMENT OF VARIOUS CROPS

WHEAT

The water requirement of six varieties of wheat, including emmer, was measured at Akron in 1912. The results arranged in order of increasing water requirement based on dry matter are as follows:

Variety of wheat	Water requirement
Turkey	364±6
Kharkov	365±6
Kubanka	394±7
Emmer	428±3
Bluestem	451±4
Spring Ghirka	457±3

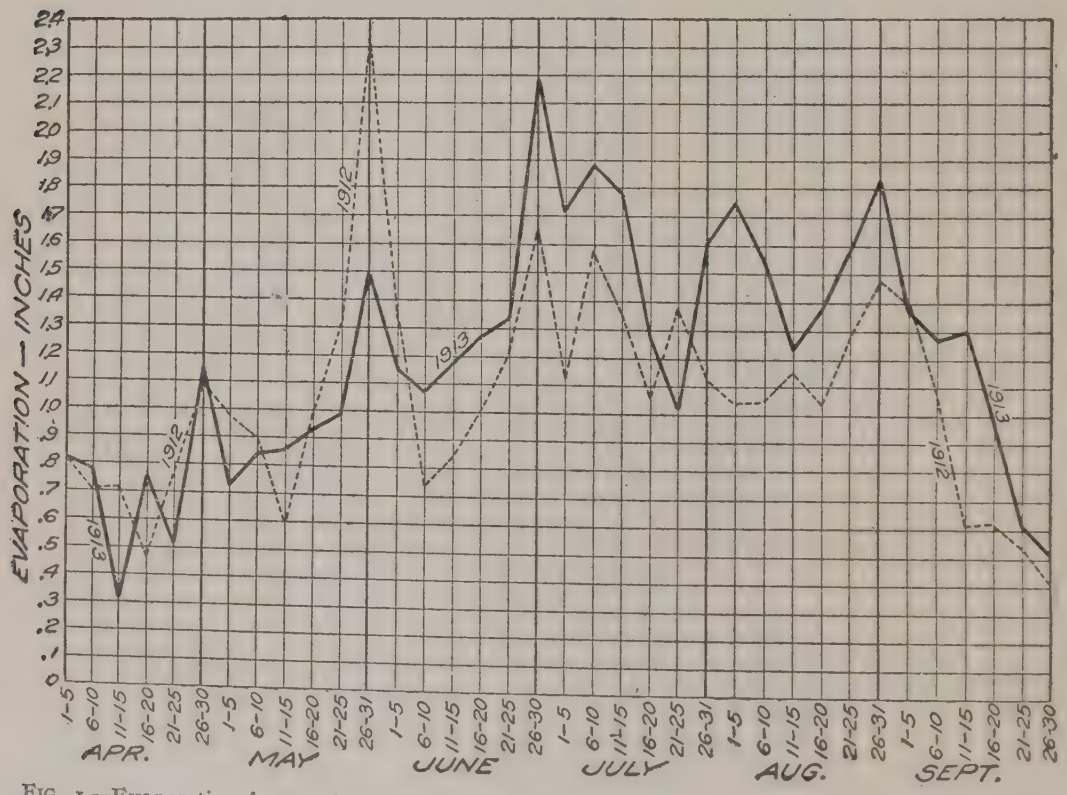

FIG. 1.—Evaporation from a free-water surface (tank) at Akron, Colo., in 1911 and 1912. Note the marked reduction in evaporation in 1912 after June 10. A volcanic eruption in Alaska occurred on June 6.

The Turkey and Kharkov varieties (Pl. III, fig. 6) were tested for the first time in 1912. These are winter varieties and were transplanted to the pots in the spring from field plats sown in the fall. They gave the same water requirement and appear to be about 10 per cent more efficient than the Kubanka (Pl. III, fig. 1), which has heretofore been the most efficient wheat tested as regards economy in the use of water. This comparison, however, ignores the small quantity of dry matter in

the plants at the time of transplanting. The three remaining varieties show somewhat greater differences than in 1911, and the order is reversed. The probable error of the water requirement of emmer in the 1911 experiments was abnormally high, so that the 1912 series (Pl. III, fig. 4, and Pl. VII, fig. 4) may be considered more nearly representative of the relative position of this crop.

The water requirement of different varieties based on grain production is as follows:

Variety of wheat	Water requirement
Emmer (including glumes)	984±18
Turkey	995±22
Kharkov	1,064±60
Kubanka	1,111±37
Emmer (without glumes)	1,243±23
Spring Ghirka	1,468±34
Bluestem	1,573±49

In order to reduce the results obtained with emmer to a basis comparable with the other varieties, the calculations should be made upon the weight of the grain without the glumes, which constitute about 21 per cent of the total weight. When this is done, it will be seen that the water requirement of the different varieties based on grain production follows the same order as when based on the production of dry matter. The Turkey wheat again gives the lowest value for the water requirement, although the Turkey, Kharkov, and Kubanka may be considered substantially in agreement when the errors of the experiment are considered. The detailed results are given in Table V.

TABLE V.—*Water requirement of different varieties of wheat at Akron, Colo., in 1912 and 1913*

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Kubanka, C. I. 1440 (*Triticum durum*), May 9 to Sept. 3	1	270.0	108.2	95.1	40	879	352
	2	252.7	88.3	97.1	35	1,099	384
	3	279.4	98.1	109.9	32	1,120	393
	4	288.8	99.7	118.6	35	1,190	411
	5	291.8	99.5	122.0	34	1,226	418
	6	261.7	92.2	106.2	35	1,152	406
Mean						1,111±37	394±7
Marvel Bluestem, C. I. 3082 (*Triticum aestivum*), May 11 to Aug. 28	31	286.2	74.2	126.3	26	1,701	441
	32	333.0	93.1	147.4	28	1,582	443
	33	310.8	87.9	145.7	28	1,658	469
	34	298.8	77.2	134.3	26	1,740	450
	35	321.4	102.9	149.5	32	1,452	465
	36	334.3	111.8	145.7	33	1,303	436
Mean						1,573±49	451±4

TABLE V.—*Water requirement of different varieties of wheat at Akron, Colo., in 1912 and 1913*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Kharkov, C. I. 1583 (*Triticum aestivum*), Apr. 27 to Aug. 28...	37	365.6	91.6	138.0	25	1,505	377
	38	347.3	131.3	122.0	38	930	351
	39	360.0	128.7	141.6	36	1,100	394
	40	384.9	138.1	135.6	40	982	353
	41	326.9	124.4	121 5	38	978	372
	42	310.7	119.2	106 1	38	890	342
Mean						1,064±60	365±6
Turkey, C. I. 1571 (*Triticum aestivum*), Apr. 27 to Aug. 1...	43	344.4	121.2	132 5	35	1,092	385
	44	328.4	112.4	120.0	34	1,070	365
	45	242.7	86.1	85.6	35	995	353
	46	308.0	119.6	106.7	39	892	346
	47	245.9	97.7	95.2	40	974	387
	48	274.4	100.8	95.4	37	946	348
Mean						995±22	364±6
Spring Ghirka, C. I. 1517 (*Triticum aestivum*), May 11 to Aug. 12	55	266.8	74.4	122.5	28	1,645	459
	56	263.3	81.0	120.6	31	1,489	458
	57	264.6	82.1	126.3	31	1,540	477
	58	275.9	90.2	125.6	33	1,393	456
	59	297.9	94.2	131.5	32	1,395	442
	60	314.7	105.2	141.6	33	1,347	450
Mean						1,468±34	457±3
Emmer, C. I. 2951 (*Triticum dicoccum*), May 11 to Aug. 12..	61	351.5	155.6	145.1	44	932	413
	62	314.7	142.3	132.5	45	930	421
	63	352.8	146.1	158.0	41	1,080	448
	64	340.5	143.8	147.8	42	1,028	434
	65	358.1	159.7	151.6	42	950	423
	66	343.0	149.0	146.3	43	982	426
Mean						984±18	428±3
1913.							
Kubanka, C. I. 1440 (*Triticum durum*), May 23 to Aug. 13...	73	294.8	106.5	150.3	36	1,411	510
	74	273.4	101.4	135.9	37	1,340	497
	75	257.0	97.1	122.4	38	1,261	476
	76	304.2	116.2	156.0	38	1,342	513
	77	253.3	93.8	121.3	37	1,293	479
	78	299.6	116.8	150.2	39	1,286	502
Mean						1,322±16	496±5

Only one variety of wheat, the Kubanka, was included in the measurements of 1913. This variety gave a water requirement 26 per cent above the 1912 ratio and 19 per cent above the 1911 ratio. The water requirement on the basis of grain production was 19 per cent higher than in 1912 and 11 per cent higher than in 1911.

OATS

The four varieties of oats employed in the water-requirement tests in 1912 were the same as those used in the 1911 experiments. The water requirement in 1912, based on the total dry matter produced, was as follows:

Variety of oats	Water requirement
Canadian	399±6
Swedish Select	423±5
Burt	449±3
Sixty-Day	491±13

The Canadian again proved to be the most efficient of the varieties tested. The differences exhibited by the first three varieties in the list are practically the same as in 1911.

Much trouble was experienced in obtaining a stand of Sixty-Day oats. The germination was very poor and a second and even a third planting failed to give a good stand, as is shown by the variations in the yield of the different pots. (Table VI.) This is, perhaps, the cause of the higher water requirement obtained for Sixty-Day oats, which in 1911 ranked next to the Canadian in efficiency.

The water requirement of the different oat varieties, based on grain production in 1912, was as follows:

Variety of oats	Water requirement
Swedish Select	1,103±18
Sixty-Day	1,172±133
Burt	1,224±55
Canadian	1,416±119

The probable error is high in all the determinations, except in the case of the Swedish Select (Pl. III, fig. 5), and the relative order of the varieties is consequently of little significance. It is, however, of interest to observe that the Canadian variety is the least efficient in the use of water from the standpoint of grain production, which is in accord with the 1911 experiments.

Swedish Select and Burt oats were also included in the 1913 measurements at Akron. On the basis of dry matter produced, the two varieties were equally efficient in the use of water. In the measurements of 1912 and 1911 these two varieties gave only slight differences, the 1911 and the 1912 results being in accord when the probable errors are considered. On the basis of grain production, the Burt was the more efficient in 1913, and the Swedish Select in 1912 and in 1911. No real differences of importance are shown in these two varieties when the measurements of the three years are considered.

TABLE VI.—*Water requirement of different varieties of oats at Akron, Colo., in 1912 and 1913*

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
		Grams.	*Grams.*	*Kilos.*	*Per cent.*		
1912.							
Sixty-Day, C. I. 165 (*Avena sativa*), May 15 to Aug. 23	67	206. 5	72. 8	119. 9	36	1, 645	580
	68	287. 7	147. 9	131. 8	51	892	458
	69	270. 9	107. 3	133. 6	40	1, 245	493
	70	93. 9	.....	41. 6	.....	.....	444
	71	274. 7	143. 4	129. 5	52	904	472
	72	248. 9	.....	124. 7	.....	.....	501
Mean						1, 172±133	491±13
Canadian, C. I. 444 (*Avena sativa*), May 17 to Sept. 16	73	227. 1	80. 9	91. 1	36	1, 125	401
	74	302. 1	118. 7	126. 7	39	1, 068	419
	75	233. 5	50. 4	89. 1	22	1, 767	382
	76	276. 0	79. 8	107. 8	29	1, 350	390
	77	158. 3	.....	59. 3	.....	.....	375
	78	250. 0	60. 5	107. 0	24	1, 769	428
Mean						1, 416±119	399±6
Burt, C. I. 293 (*Avena sativa*), May 15 to Aug. 23	79	352. 8	138. 8	153. 5	39	1, 106	435
	80	339. 9	110. 5	158. 4	33	1, 433	466
	81	299. 5	93. 8	135. 8	31	1, 448	453
	82	344. 5	132. 9	150. 9	39	1, 135	438
	83	351. 5	147. 8	160. 3	42	1, 085	456
	84	363. 8	143. 3	162. 7	39	1, 135	447
Mean						1, 224±55	449±3
Swedish Select, C. I. 134 (*Avena sativa*), May 17 to Aug. 23	85	402. 2	167. 7	167. 2	42	997	416
	86	395. 7	145. 9	171. 5	37	1, 176	434
	87	409. 5	155. 9	171. 2	38	1, 098	418
	88	412. 2	152. 9	164. 0	37	1, 073	398
	89	389. 8	145. 9	169. 7	37	1, 163	435
	90	366. 8	144. 5	161. 0	39	1, 113	439
Mean						1, 103±18	423±5
1913.							
Swedish Select, C. I. 134 (*Avena sativa*), May 23 to Aug. 1	79	265. 5	84. 2	171. 4	32	2, 035	646
	80	250. 1	83. 0	164. 2	33	1, 979	656
	81	262. 4	77. 4	158. 5	29	2, 049	604
	82	296. 7	106. 9	175. 3	36	1, 640	591
	83	286. 1	101. 4	174. 3	35	1, 719	609
	84	291. 9	95. 0	174. 0	33	1, 831	596
Mean						1, 876±55	617±9
Burt, C. I. 293 (*Avena sativa*), May 23 to July 25	85	243. 7	93. 4	148. 8	38	1, 594	611
	86	245. 6	101. 8	151. 1	41	1, 484	616
	87	240. 4	87. 6	156. 5	36	1, 787	650
	88	255. 5	89. 3	157. 0	35	1, 758	614
	89	250. 0	94. 7	155. 7	38	1, 644	623
	90	254. 9	94. 5	149. 3	37	1, 580	586
Mean						1, 641±33	617±5

BARLEY

Barley is the most uniform in water requirement of the small-grain crops which the writers have tested. The four varieties grown at Akron in 1912 showed only slight differences in their water requirement. The results obtained, based upon the production of dry matter, were as follows:

Variety of barley	Water requirement
Beardless	403±8
Beldi	416±4
White Hull-less	439±1
Hannchen	443±3

These same varieties were also tested at Akron in 1911 and were found to be in practical agreement as regards their relative water requirement. The mean value of the water requirement was 27 per cent higher in 1911 than in 1912.

The results obtained with barley when the water requirement is based on grain production are less uniform than when the total dry matter is employed. Reference to Table VII will show that this is often due to a single pot which for some reason fails to set grain as abundantly as the rest of the series. The Beldi, a dwarf variety, showed the highest efficiency in the use of water in grain production. The White Hull-less (Pl. III, fig. 2) has a water requirement slightly above the other varieties, even when a correction is made for the naked character of the grain.

TABLE VII.—*Water requirement of different varieties of barley at Akron, Colo., in 1912*

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Hannchen, C. I. 531 (*Hordeum distichon*), May 16 to Aug. 28	91	301.5	141.7	138.1	47	974	458
	92	282.9	116.6	127.7	41	1,095	452
	93	281.1	103.5	124.2	37	1,200	442
	94	300.3	137.1	133.3	46	972	444
	95	309.2	145.5	134.9	47	926	436
	96	308.5	152.0	130.9	49	860	424
Mean						1,005±36	443±3
Beldi, C. I. 190 (*Hordeum vulgare*), May 16 to Aug. 12	97	230.5	101.5	93.9	44	925	407
	98	240.1	107.0	98.5	45	920	410
	99	243.0	109.2	99.5	45	910	409
	100	189.4	81.3	82.1	43	1,010	434
	101	223.8	101.6	97.0	45	954	434
	102	236.5	103.1	95.5	44	926	404
Mean						941±10	416±4
White Hull-less, C. I. 595 (*Hordeum vulgare*), May 16 to Aug. 12	103	271.2	95.3	119.7	35	1,256	441
	104	276.0	97.1	121.4	35	1,250	440
	105	273.1	95.6	119.4	35	1,249	437
	106	268.8	92.9	119.7	35	1,289	445
	107	273.8	99.5	118.4	36	1,190	433
	108	299.7	110.0	131.6	37	1,197	439
Mean						1,239±11	439±1

TABLE VII.—*Water requirement of different varieties of barley at Akron, Colo., in 1912*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Beardless, C. I. 716 (*Hordeum vulgare*), May 16 to Aug. 23...	109	112.0	20.7	49.2	18	.....	439
	110	144.1	21.9	56.1	15	.....	389
	111	276.3	135.7	111.6	49	823	408
	112	227.5	92.2	93.8	40	1,017	412
	113	210.5	63.9	87.2	30	1,364	414
	114	291.3	120.2	103.7	41	862	356
Mean.....	.....	.....	.....	.....	.....	1,017±83	403±8

RYE

The measurement of the water requirement of spring rye at Akron in 1911 showed a surprisingly high figure—54 per cent above that of Kubanka wheat. The 1912 measurements (Table VIII) gave 496±9 for the water requirement of rye when based on dry matter and 1,802±62 when based on grain production. The 1912 (dry matter) ratio is thus about 26 per cent above Kubanka wheat, a marked increase in the relative efficiency in comparison with the 1911 ratio. In fact, rye exhibited the greatest reduction in water requirement of all the crops tested in 1912.

A consideration of the water requirement of crops grown out of season in 1911 showed that rye was unusually efficient during the cool fall period. This result as well as the increase in efficiency in 1912 suggests that rye may be unusually responsive to climatic conditions and that it is relatively better adapted to low temperature than the other small grains.

TABLE VIII.—*Water requirement of rye at Akron, Colo., in 1912*

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Rye, spring, C. I. 73 (*Secale cereale*), May 16 to Aug. 23.....	115	210.6	57.8	96.2	27	1,664	457
	116	216.4	65.1	118.8	30	1,825	549
	117	179.2	52.6	84.3	29	1,603	470
	118	248.6	65.8	123.3	26	1,874	496
	119	234.5	54.7	120.1	23	2,195	512
	120	254.1	75.6	124.6	30	1,649	490
Mean.....	.....	.....	.....	.....	.....	1,802±62	496±9

RICE

Rice was grown for the first time at Akron in 1912. The crop was slow in becoming established, and the growth period was relatively long. No grain was produced. The water requirement based on dry matter was 519±13 (Table IX). It thus appears that rice, although a crop normally grown with an abundant water supply and in a relatively humid climate, is about as efficient in the use of water as rye. Its relative position might be materially changed if the tests were made in a warmer climate.

Rice was also included in the 1913 measurements (Pl. VII, fig. 3). The stand was good and the growth was uniform and luxurious, but the season was too short to produce grain. The water requirement in 1913 was 744±17, or 43 per cent higher than in 1912.

TABLE IX.—*Water requirement of rice at Akron, Colo., in 1912 and 1913*

Plant and period of growth.	Pot No.	Dry matter.	Water.	Water requirement based on dry matter.
1912.		*Grams.*	*Kilos.*	
Rice, Honduras, C. I. 1643 (*Oryza sativa*), May 27 to Sept. 23	151	248.4	133.5	538
	152	253.1	141.2	558
	153	232.5	127.4	548
	154	168.9	90.5	536
	155	194.0	94.8	488
	156	225.6	100.0	445
Mean				519±13
1913.				
Rice, Honduras, C. I. 1643, June 12 to Sept. 16	157	276.7	218.2	790
	158	261.7	211.5	809
	159	230.2	176.9	768
	160	274.8	186.6	680
	161	294.8	204.0	692
	162	298.7	215.8	722
Mean				744±17

FLAX

Flax was included in the water-requirement measurements at Akron for the first time in 1913. Its water requirement was found to be very high, 905±25 based on dry matter and 2,835±52 when based on seed production. It will thus be seen to have a water requirement as high or higher than any of the legumes tested in 1913. This is in accord with the measurements made by Leather (1911, p. 270) in India, in which flax was exceeded in water requirement only by chick-peas and rice. At Akron in 1913 flax required 22 per cent more water than rice. The detailed results are given in Table X.

TABLE X.—*Water requirement of flax at Akron, Colo., in 1913*

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1913.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Flax, North Dakota, No. 155 (*Linum usitatissimum*), June 3 to Sept. 1....	127	189.0	64.9	184.8	34	2,845	978
	128	154.7	53.0	143.4	34	2,704	927
	129	209.3	77.2	210.6	37	2,728	1,006
	130	93.0	23.9	76.3	26	3,190	811
	131	116.7	32.5	93.9	28	2,886	804
	132	187.8	63.7	169.2	34	2,658	902
Mean.....	.....	.....	.....	.....	.....	2,835±52	905±25

SUGAR BEETS

The water requirement of the sugar beet was again measured at Akron in 1912. The ratio 321±8 was obtained on the basis of total dry matter and 524±23 on the basis of the dry root (Table XI). This is about 15 per cent below the 1911 value, a reduction in water requirement similar to that shown by the other crops tested during the two seasons. The sugar beet is an efficient plant in the use of water, being the equal of the corn group in this respect.

TABLE XI.—*Water requirement of sugar beets at Akron, Colo., in 1912*

Plant and period of growth.	Pot No.	Dry matter.	Roots.	Water.	Roots.	Water requirement based on—	
						Dry roots.	Total dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Sugar beet (*Beta vulgaris*), June 9 to Oct. 12.....	169	257.4	189.7	76.0	74	401	295
	170	175.0	93.6	55.7	53	595	318
	171	173.3	100.0	49.6	58	496	286
	172	163.6	107.3	52.5	66	489	321
	173	196.8	123.0	68.7	63	558	349
	174	164.5	97.7	58.9	60	603	358
Mean.....	.....	.....	.....	.....	.....	524±23	321±8

COTTON

Cotton was tested at Akron for the first time in 1912. Numerous bolls set, though none opened. The plants grew slowly during the first part of the season, owing probably to the cool nights. The observed water-requirement ratio was 488±14 (Table XII).

TABLE XII.—*Water requirement of cotton at Akron, Colo., in 1912 and 1913*

Plant and period of growth.	Pot No.	Dry matter.	Water.	Water requirement based on dry matter.
1912.		*Grams.*	*Kilos.*	
Cotton, Triumph (*Gossypium hirsutum*), July 19 to Sept. 21	181	38. 9	19. 2	494
	182	32. 0	18. 5	578
	183	67. 4	27. 1	402
	184	61. 4	28. 2	459
	185	51. 6	25. 2	488
	186	62. 4	31. 5	505
Mean				488±14
1913.	163	237. 5	172. 4	726
	164	184. 3	115. 6	627
Cotton, Triumph, May 29 to Sept. 16	165	247. 4	166. 1	671
	166	249. 6	160. 1	642
	167	161. 4	106. 4	660
	168	187. 0	115. 4	617
Mean				657±11

The same variety was also included in the 1913 measurements. (Pl. VII, fig. 1.) The planting was made earlier, and a much larger growth was obtained. The water requirement was 657±11, or about one-third higher than in 1912. In this connection it should be stated that at Akron cotton is far north of its natural range, which may have increased its relative water requirement.

CORN AND TEOSINTE

Six varieties of corn (*Zea mays*) were tested at Akron in 1912 (Pl. VII, fig. 5). Three of these varieties, Northwestern Dent (Pl. IV, fig. 1), Iowa Silvermine, and Esperanza, had also been used in the 1911 experiments. The three new varieties were furnished by Mr. G. N. Collins, of the Bureau of Plant Industry, and represent widely different strains. The Hopi variety (Pl. IV, fig. 2) is grown by the Hopi Indians in northwestern New Mexico (Collins, 1914); China White is a variety from near Shanghai, China; while Laguna was originally from the State of Chihuahua, Mexico. The water requirement of each variety tested in 1912, based on the production of dry matter, is as follows:

Variety of corn	Water requirement
Esperanza	239±3
Northwestern Dent	280±10
Hopi	285±7
Laguna	295±6
Iowa Silvermine	302±7
China White	315±7

The Esperanza, as in 1911, leads all the varieties, so far as efficiency in the production of dry matter is concerned, and ranks with the sor-

ghums in this respect. The differences exhibited by the remaining varieties are without significance when the limitations imposed by the probable errors are considered, although the quick-maturing Northwestern Dent and Hopi varieties appear to be slightly more efficient than the others. The detailed data are given in Table XIII. The pollination was not adequate to give representative grain yields.

Five varieties of corn and one of teosinte were included in the 1913 measurements. The water requirement of each variety, based on the production of dry matter, is as follows:

Variety of corn or teosinte	Water requirement
Indian Flint corn	342±5
Hopi corn	350±8
Teosinte, Durango	390±11
Northwestern Dent corn	399±12
Bloody Butcher corn	405±7
China White corn	415±4

The most efficient varieties were the Indian Flint (Pl. VI, fig. 5), a small variety grown by the Indians of northern Michigan, and the Hopi, another dwarf Indian variety. Teosinte, Northwestern Dent corn, and Bloody Butcher, a local variety of corn grown near Wray, Colo., showed only slight differences. The China White, as in 1912, proved to be the least efficient of all the varieties tested, having a water requirement 20 per cent above that of the Indian varieties.

In 1912 the water requirement of the Northwestern Dent corn was in practical agreement with that of the Hopi, while in 1913 the Hopi gave a considerable lower value. The China White required 32 per cent more water in 1913 than in 1912; the Northwestern Dent, 42 per cent; and the Hopi, 23 per cent.

The water requirement of certain corn hybrids was also measured at Akron in 1912 and 1913. The mean water requirement of each strain has been included in the tables in the summary (Tables XXIX to XXXII).

TABLE XIII.—*Water requirement of different varieties of corn and teosinte at Akron, Colo., in 1912 and 1913*

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Corn, Northwestern Dent (*Zea mays*), June 9 to Sept. 16...	277	299.0	18.1	102.6	6	.....	343
	278	344.2	53.9	100.4	16	.....	292
	279	368.5	66.1	105.5	18	.....	286
	280	649.0	234.4	161.1	36	.....	248
	281	440.0	101.5	111.2	23	.....	253
	282	491.0	117.1	126.1	24	.....	257
Mean.....	.....	.....	.....	.....	.....	.....	280±10

TABLE XIII.—*Water requirement of different varieties of corn and teosinte at Akron, Colo., in 1912 and 1913*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Corn, Iowa Silvermine (*Zea mays*), June 8 to Sept. 26	283	596.0	.....	167.9	.....	.....	282
	284	403.7	.....	129.9	.....	.....	322
	285	399.7	.....	133.4	.....	.....	334
	286	441.5	.....	129.6	.....	.....	294
	287	477.0	.....	135.6	.....	.....	284
	288	437.2	.....	128.9	.....	.....	295
Mean	.....	.....	.....	.....	.....	.....	302±7
Corn, Hopi (*Zea mays*), June 12 to Sept. 26	295	433.0	.....	132.7	.....	.....	307
	296	519.5	.....	140.3	.....	.....	270
	297	516.8	.....	131.1	.....	.....	254
	298	415.8	.....	113.4	.....	.....	273
	299	330.9	.....	100.2	.....	.....	303
	300	364.7	.....	111.3	.....	.....	305
Mean	.....	.....	.....	.....	.....	.....	285±7
Corn, China White (*Zea mays*), June 12 to Sept. 26	265	243.5	.....	84.3	.....	.....	346
	266	577.0	.....	184.6	.....	.....	320
	267	319.0	.....	97.1	.....	.....	304
	268	524.5	.....	173.9	.....	.....	331
	269	660.9	.....	179.6	.....	.....	272
	270	401.5	.....	126.1	.....	.....	314
Mean	.....	.....	.....	.....	.....	.....	315±7
Corn, Laguna (*Zea mays*), July 2 to Sept. 26	289	376.6	.....	112.4	.....	.....	298
	290	261.2	.....	83.8	.....	.....	321
	291	268.9	.....	84.0	.....	.....	313
	292	448.1	.....	124.4	.....	.....	278
	293	457.4	.....	127.8	.....	.....	279
	294	429.2	.....	119.4	.....	.....	278
Mean	.....	.....	.....	.....	.....	.....	295±6
Corn, Esperanza (*Zea mays*), June 12 to Sept. 26	301	492.3	.....	114.3	.....	.....	232
	302	574.7	.....	133.7	.....	.....	233
	303	563.7	.....	141.7	.....	.....	252
	304	510.7	.....	122.7	.....	.....	240
Mean	.....	.....	.....	.....	.....	.....	239±3
1913.							
Corn, Bloody Butcher (*Zea mays*), June 7 to Sept. 13	247	411.5	.....	174.1	.....	.....	423
	248	485.4	.....	201.5	.....	.....	415
	249	456.6	.....	188.6	.....	.....	413
	250	501.9	.....	193.0	.....	.....	385
	251	499.4	.....	183.6	.....	.....	368
	252	456.8	.....	195.4	.....	.....	428
Mean	.....	.....	.....	.....	.....	.....	405±7
Corn, Indian Flint (*Zea mays*), June 7 to Aug. 27	253	333.4	123.4	114.9	37	931	345
	254	397.9	163.3	130.5	41	800	328
	255	380.6	161.7	120.6	42	746	317
	256	292.0	101.6	108.2	35	1,064	370
	257	335.1	135.2	118.7	40	878	354
	258	380.3	182.6	128.6	48	704	338
Mean	.....	.....	.....	.....	.....	854±39	342±5

TABLE XIII.—*Water requirement of different varieties of corn and teosinte at Akron, Colo., in 1912 and 1913*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1913.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Corn, Northwestern Dent (*Zea mays*), June 7 to Sept. 6....	283	351.2	.....	153.7	.....	.....	436
	284	392.3	131.6	142.9	34	1,086	364
	285	336.6	.....	136.6	.....	.....	406
	286	285.9	.....	128.7	.....	.....	451
	287	389.9	117.5	141.4	30	1,203	363
	288	382.0	100.0	143.4	26	1,434	375
Mean.....	.....	.....	.....	.....	.....	1,241±77	399±12
Corn, Hopi (*Zea mays*), June 14 to Sept. 16..	313	346.7	.....	118.8	.....	.....	343
	314	400.0	.....	135.8	.....	.....	340
	315	472.5	.....	170.2	.....	.....	360
	316	417.1	.....	147.0	.....	.....	352
	317	405.3	.....	163.4	.....	.....	403
	318	619.6	.....	185.4	.....	.....	300
Mean.....	.....	.....	.....	.....	.....	.....	350 ±8
Corn, China White (*Zea mays*), June 7 to Sept. 16.....	301	554.9	.....	228.2	.....	.....	411
	302	487.5	.....	210.4	.....	.....	432
	303	492.6	.....	202.3	.....	.....	411
	304	589.2	.....	228.1	.....	.....	387
	305	478.7	.....	198.7	.....	.....	415
	306	523.1	.....	226.6	.....	.....	433
Mean.....	.....	.....	.....	.....	.....	.....	415 ±4
Teosinte, Durango (*Euchlaena mexicana*), June 14 to Sept. 16.....	289	616.4	.....	234.7	.....	.....	380
	290	534.5	.....	211.6	.....	.....	396
	291	624.5	.....	194.0	.....	.....	310
	292	567.3	.....	231.5	.....	.....	408
	293	520.0	.....	214.4	.....	.....	412
	294	421.4	.....	183.2	.....	.....	435
Mean.....	.....	.....	.....	.....	.....	.....	390±11

SORGHUM

The investigation of the water requirement of the sorghums (Table XIV) is of special interest, owing to the marked efficiency exhibited by this group of plants in the use of water. The eight varieties grown at Akron in 1912, together with the water requirement based on the production of dry matter, follow:

Variety of sorghum	Water requirement
Brown kaoliang	223±1
Red Amber	237±4
Minnesota Amber	239±2
Milo	249±3
White durra	255±3
Blackhull kafir	259±5
Dwarf milo	273±4
Sudan grass	359±2

The Brown kaoliang gave the lowest water requirement. Red Amber and Minnesota Amber, forage varieties of sorghum (Pl. IV, figs. 4 and 5), gave practically the same ratio, which is but slightly higher than Brown kaoliang.

TABLE XIV.—*Water requirement of different sorghums at Akron, Colo., in 1912 and 1913*

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Red Amber, S. P. I. 17543 (*Andropogon sorghum*), June 9 to Sept. 27	253	400.5	43.8	103.1	11	2,351	258
	254	541.7	41.5	133.8	8	3,221	247
	255	592.0	58.1	135.5	10	2,331	229
	256	660.5	95.4	144.8	14	1,518	219
	257	564.0	44.3	135.1	8	3,050	240
	258	714.2	93.5	161.0	13	1,722	226
Mean						2,366±194	237±4
Minnesota Amber, A. D. I. 341–13 (*Andropogon sorghum*), June 9 to Sept. 26	247	666.6	272.6	151.2	41	554	227
	248	370.6	139.9	89.6	38	645	242
	249	543.0	218.4	128.1	40	586	236
	250	461.3	168.1	110.6	37	658	240
	251	456.6	195.7	110.3	43	564	242
	252	425.3	163.3	103.6	38	634	244
Mean						607±15	239±2
Milo, Dwarf, S. P. I. 24970 (*Andropogon sorghum*), June 9 to Sept. 27	217	434.5	83.0	115.1	19	1,387	265
	218	370.2	79.9	102.0	22	1,276	275
	219	334.3	55.3	90.6	17	1,638	271
	220	403.7	78.5	105.7	19	1,347	262
	221	420.1	84.5	110.2	20	1,304	262
	222	301.2	64.5	91.8	21	1,422	305
Mean						1,396±34	273±4
Milo, S. P. I. 24960 (*Andropogon sorghum*), June 9 to Sept. 27	223	475.4	60.0	125.6	13	2,092	264
	224	440.4	85.7	103.7	19	1,210	235
	225	472.5	77.9	114.5	17	1,470	242
	226	488.9	114.0	123.3	23	1,081	252
	227	499.9	116.0	123.0	23	1,060	246
	228	509.9	79.6	128.8	16	1,618	253
Mean						1,422±115	249±3
Durra, White, S. P. I. 24997 (*Andropogon sorghum*), June 9 to Sept. 26	235	506.9	138.7	129.3	27	925	255
	236	568.9	142.7	142.1	25	996	250
	237	432.9	85.6	106.7	20	1,246	247
	238	384.6	63.5	105.1	17	1,656	273
	239	406.2	78.1	99.4	19	1,273	245
	240	449.6	108.6	115.9	24	1,067	258
Mean						1,194±75	255±3
Kaoliang, Brown, S. P. I. 24993 (*Andropogon sorghum*), June 9 to Sept. 26	241	588.9	144.8	134.0	25	925	228
	242	411.1	108.8	94.8	26	870	230
	243	548.9	125.3	120.5	23	962	220
	244	556.0	126.0	123.8	23	982	223
	245	526.0	171.0	116.8	33	683	222
	246	571.8	108.7	123.9	19	1,140	217
Mean						927±38	223±1

TABLE XIV.—*Water requirement of different sorghums at Akron, Colo., in 1912 and 1913*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Kafir, Blackhull, S. P. I. 24975 (*Andropogon sorghum*), June 9 to Sept. 27....	229	247.0	.....	72.1	.....	.....	292
	230	409.1	.....	108.4	.....	.....	265
	231	494.5	.....	119.3	.....	.....	241
	232	413.0	.....	101.8	.....	.....	246
	233	363.3	.....	91.9	.....	.....	253
	234	377.1	.....	97.4	.....	.....	258
Mean.....	.....	.....	.....	.....	.....	.....	259±5
Sudan grass, S. P. I. 25017 (*Andropogon sorghum aethiopicus*), first crop, May 28 to July 26.....	211	190.6	.....	60.8	.....	.....	319
	212	209.4	.....	65.8	.....	.....	314
	213	181.8	.....	59.4	.....	.....	326
	214	205.8	.....	63.4	.....	.....	308
	215	215.9	.....	63.8	.....	.....	296
	216	222.6	.....	68.6	.....	.....	308
Mean.....	.....	.....	.....	.....	.....	.....	312±3
Sudan grass, S. P. I. 25017, second crop, July 26 to Sept. 6....	211	106.7	.....	46.1	.....	.....	432
	212	94.0	.....	43.5	.....	.....	463
	213	95.6	.....	44.6	.....	.....	467
	214	106.5	.....	45.9	.....	.....	431
	215	91.0	.....	42.9	.....	.....	472
	216	88.0	.....	43.0	.....	.....	489
Mean.....	.....	.....	.....	.....	.....	.....	459±7
Sudan grass, S. P. I. 25017, combined crop, May 28 to Sept. 6.....	211	297.3	.....	106.9	.....	.....	359
	212	303.4	.....	109.3	.....	.....	360
	213	277.4	.....	104.0	.....	.....	375
	214	312.3	.....	109.3	.....	.....	350
	215	306.9	.....	106.7	.....	.....	348
	216	310.6	.....	111.6	.....	.....	360
Mean.....	.....	.....	.....	.....	.....	.....	359±2
1913.							
Sorghum, Minnesota Amber, A. D. I. 341–13 (*Andropogon sorghum*), June 14 to Sept. 15.....	265	557.8	213.0	164.5	38	772	295
	266	585.0	256.9	181.4	44	706	310
	267	591.8	235.4	177.4	40	754	299
	268	677.5	263.0	203.1	39	773	301
	269	577.9	204.0	167.5	35	821	290
	270	643.0	150.7	189.7	23	.....	295
Mean.....	.....	.....	.....	.....	.....	765±12	298±2
Sorghum, Red Amber, S. P. I. 17543 (*Andropogon sorghum*), June 7 to Sept. 15...	277	689.7	209.4	200.5	30	958	291
	278	670.7	190.6	196.0	28	1,028	292
	279	636.2	160.2	187.9	25	1,172	295
	280	644.1	165.6	193.9	26	1,170	301
	281	682.3	187.4	200.5	23	1,070	294
	282	749.7	187.8	225.2	25	1,199	301
Mean.....	.....	.....	.....	.....	.....	1,100±31	296±1

The least efficient variety tested in the sorghum group is Sudan grass (Pl. V, fig. 1), a forage plant which has recently received considerable attention in the southern Great Plains. Only one year's measurements are available for Sudan grass, but the results so far indicate that it is not the equal of other well-known varieties of sorghum in efficiency in the use of water. Sudan grass required 40 per cent more water than Brown kaoliang for the production of the first crop. The second crop was light at Akron and had a much higher water requirement. On the basis of the two cuttings combined, the water requirement of Sudan grass was 62 per cent higher than Brown kaoliang. As a forage crop, however, the shorter and more slender stalks of Sudan grass may offset the disadvantage of its higher water requirement.

In the production of grain the Minnesota Amber[1] variety gave the lowest water requirement ratio so far recorded for a sorghum crop, viz, 607 ± 15. The Minnesota Amber produced a pound of grain at Akron in 1912 with less water than was required by alfalfa in the production of a pound of hay. The high water requirement for grain production in Red Amber sorghum, Dwarf milo, milo, and White durra (Pl. IV, fig. 3) is largely due to an attack of aphids, which caused many of the flowers to fail to produce seed. The parasites were killed by spraying early enough to prevent any serious reduction in total growth.

The 1913 water requirement measurements of sorghum were confined to two varieties, Red Amber and Minnesota Amber, both of which were included in the 1912 measurements. The two varieties gave in 1913 practically identical water-requirement ratios—namely, 296 ± 1 and 298 ± 2. The results from individual pots were in excellent agreement as indicated by the small probable error. A similar agreement was observed in 1912. Each variety in 1913 showed an increase of 25 per cent in the water requirement as compared with 1912.

A series of water-requirement measurements were made at Amarillo, Tex., in 1913, for the purpose of determining the influence of climatic environment on the water requirement. These measurements also included a number of sorghum varieties, the water requirement of which had never before been determined. Plants have a higher water requirement at Amarillo than at Akron, so that measurements of different plants at the two stations are not directly comparable. The water requirement of Red Amber and Minnesota Amber sorghum was measured at both stations in 1913, and the ratio of these measurements affords a means for reducing the Amarillo values to the basis of the Akron measurements. The mean water requirement of these two varieties at Akron was 85 per cent of that at Amarillo. The Amarillo water-requirement measurements as given in Table XVI have been reduced accordingly

[1] This variety was represented by a strain selected for its drought resistance by Mr. A. C. Dillman, of the Office of Alkali and Drought Resistant Plant Investigations.

for comparison with the Akron measurements. The computed values for Akron are given in Table XV.

TABLE XV.—*Observed water requirement of varieties of sorghum at Amarillo, Tex., and computed water requirement for Akron, Colo., in 1913*

Variety.	Observed water requirement at Amarillo.	Computed water requirement for Akron.
Dwarf Blackhull kafir	335±3	285±3
White kafir	349±4	297±4
Early Blackhull kafir	356±15	302±13
White milo	373±3	317±3
Kafir×durra	378±5	321±5
Feterita	380±4	323±4

It will be noted (Table XV) that Dwarf Blackhull kafir and Minnesota Amber sorghum were the most efficient in the use of water of the eight varieties of sorghum tested at Amarillo in 1913. The least efficient was feterita. The kafir×durra hybrid had practically the same water requirement as feterita. The latter has been extensively featured recently as a drought-resistant crop particularly adapted to the Southwest. It does not appear, however, that its drought-resistant qualities are ascribable to an efficiency in the use of water, this variety being the highest in water requirement of all the sorghums tested at Amarillo in 1913. Vinall and Ball (1913, p. 27) have suggested that the success of feterita during recent dry years has been due to a thin stand resulting in part from poor germination. When grown under identical conditions as to stand, it showed no greater drought resistance than milo or kafir.

TABLE XVI.—*Water requirement of sorghum at Amarillo, Tex., in 1913*

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1913.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Sorghum, Red Amber, S. P. I. 17543 (*Andropogon sorghum*), May 15 to Aug. 20...	43	717.4	159.3	267.8	22	1,680	373
	44	682.2	137.9	262.4	20	1,904	385
	45	723.6	205.9	268.0	28	1,302	371
	46	701.9	117.4	256.2	17	2,182	365
	47	741.3	197.0	270.7	27	1,374	365
	48	768.7	208.7	272.9	27	1,306	355
Mean						1,625±112	369±3
Sorghum, Minnesota Amber, A. D. I. 341–13 (*Andropogon sorghum*), May 15 to Aug. 8	49	590.2	233.2	196.2	39	841	332
	50	588.3	295.4	196.6	50	666	334
	51	612.7	291.9	199.7	48	685	326
	52	646.0	308.9	208.1	48	674	322
	53	577.1	264.5	201.0	46	760	348
	54	649.5	288.9	211.1	44	731	325
Mean						726±19	331±3

TABLE XVI.—*Water requirement of sorghum at Amarillo, Tex., in 1913*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1913.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Milo, White, C. I. 365 (*Andropogon sorghum*), June 7 to Aug. 22	61	464.5	80.4	173.5	17	2,159	374
	62	481.1	107.3	166.3	22	1,550	346
	63	453.0	75.6	171.9	17	2,272	380
	64	470.0	108.4	176.4	23	1,626	375
	65	449.3	79.6	170.9	18	2,148	380
	66	471.1	112.2	179.6	24	1,601	381
Mean						1,893±113	373±3
Kafir, Early Blackhull, C. I. 472 (*Andropogon sorghum*), June 11 to Sept. 16	67	587.4	182.7	198.6	31	1,087	338
	68	577.8	178.6	207.3	31	1,160	359
	69	599.4	235.7	197.6	39	839	330
	70	530.6	193.4	169.8	36	878	320
	71	440.2	220.2	206.9	50	940	470
	72	603.4	199.8	192.1	33	962	319
Mean						978±37	356±15
Kafir, Dwarf Blackhull, C. I. 340 (*Andropogon sorghum*), June 11 to Sept. 16	73	592.5	254.0	190.8	43	752	322
	74	621.6	276.1	200.0	44	724	322
	75	562.7	205.2	191.5	36	934	340
	76	586.7	260.0	197.9	44	761	337
	77	544.8	195.6	191.7	36	980	352
	78	559.3	152.7	187.0	27	1,223	334
Mean						896±57	335±3
Kafir, White, C. I. 370 (*Andropogon sorghum*), June 11 to Sept. 22	79	555.3	185.9	201.7	33	1,086	363
	80	546.2	106.4	200.1	19	[a] 1,882	366
	81	571.8	210.8	198.5	37	942	347
	82	584.2	248.7	198.0	43	796	339
	83	569.6	221.0	194.3	39	879	341
	84	579.6	228.3	195.9	39	858	338
Mean						912±34	349±4
Kafir×durra, hybrid 198–15–3 (*Andropogon sorghum*) June 11 to Sept. 22	85	552.0	226.5	200.1	41	884	363
	86	538.4	215.3	193.1	40	898	358
	87	539.7	217.6	199.1	40	916	369
	88	515.0	206.6	194.7	40	942	378
	89	510.7	179.5	197.1	35	1,098	386
	90	471.5	158.1	194.0	23	1,226	411
Mean						994±42	378±5
Feterita, C. I. 182 (*Andropogon sorghum*) June 11 to Sept. 18	91	547.8	181.3	212.1	33	1,170	387
	92	585.4	235.8	216.0	40	916	369
	93	619.3	256.9	226.9	41	884	366
	94	531.1	210.9	206.1	41	979	388
	95	562.8	210.2	208.5	37	992	370
	96	512.0	191.3	203.8	37	1,064	398
Mean						1,001±29	380±4

[a] Omitted in computing the mean.

MILLET AND PROSO

These plants are remarkable in that they outrank all others so far tested as regards efficiency in the use of water (Table XVII). The four varieties grown at Akron in 1912 gave the following water requirement, based on the production of dry matter:

Variety of millet or proso	Water requirement
Kursk millet	187±2
Voronezh proso	206±1
Tambov proso	208±1
German millet	248±7

Kursk millet (Pl. V, fig. 3) represented by a strain developed by Mr. A. C. Dillman, of the Office of Alkali and Drought Resistant Plant Investigations, gave the lowest water requirement so far recorded for any crop at Akron. The two prosos, Tambov and Voronezh (Pl. V, fig. 2), have a water requirement about 10 per cent higher than the Kursk, while German millet is 33 per cent higher than the Kursk. Aside from the German millet, all of the varieties tested have a water requirement distinctly below the best of the sorghums, the group ranking next in efficiency.

TABLE XVII.—*Water requirement of different millets and prosos at Akron, Colo., in 1912 and 1913*

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Millet, Kursk, S. P. I. 34771 (*Chaetochloa italica*), June 9 to Aug. 20	205	190. 7	66. 1	36. 7	35	555	192
	206	320. 0	119. 1	58. 9	37	494	184
	207	241. 7	102. 6	44. 1	42	430	182
	208	192. 5	78. 4	36. 1	41	461	188
	209	178. 9	66. 5	32. 6	37	490	182
	210	332. 6	139. 3	65. 0	42	467	195
Mean						483±11	187±2
Millet, German, S. P. I. 26845 (*Chaetochloa italica*), July 2 to Sept. 23	187	115. 1	.....	27. 3	.....	.....	237
	188	202. 6	.....	46. 7	.....	.....	231
	189	140. 0	.....	33. 9	.....	.....	242
	190	350. 3	.....	82. 4	.....	.....	235
	191	92. 5	.....	22. 4	.....	.....	242
	192	80. 5	.....	24. 2	.....	.....	301
Mean						.....	248±7
Proso, Tambov, S. D. 366, Akron, 366–1–10 (*Panicum miliaceum*), June 8 to Aug. 12	193	255. 3	111. 0	54. 8	43	494	214
	194	342. 9	162. 4	68. 0	47	419	198
	195	190. 0	80. 7	39. 7	42	492	209
	196	317. 6	140. 2	66. 5	44	474	209
	197	336. 2	142. 8	70. 1	43	491	208
	198	282. 7	112. 6	58. 9	40	523	208
Mean						482±9	208±1

TABLE XVII.—*Water requirement of different millets and prosos at Akron, Colo., in 1912 and 1913*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Proso, Voronezh, C. I. 16 (*Panicum miliaceum*), June 5 to Aug. 20	199	238.7	114.2	48.9	48	428	205
	200	296.1	143.7	61.2	48	426	207
	201	281.9	135.6	56.8	48	419	201
	202	298.5	149.5	59.9	50	401	201
	203	256.1	119.4	53.7	47	450	210
	204	273.0	133.2	57.0	49	428	209
Mean						425±4	206±1
1913.							
Kursk, S. P. I. 34771 (*Chaetochloa italica*), June 14 to Aug. 26	259	174.8	37.1	48.9	21	1,318	280
	260	281.7	76.1	80.7	27	1,060	286
	261	175.4	71.6	50.5	41	705	288
	262	183.3	31.9	51.3	17	.....	280
	263	250.6	33.4	66.3	13	.....	265
	264	201.8	74.0	63.5	37	858	315
Mean						.....	286±4

In grain production the millets make a remarkable showing, the prosos leading in this respect. Measurements of the water requirement of the three varieties, based on grain production, gave the following results:

Variety of millet or proso	Water requirement
Voronezh proso	425±4
Tambov proso	482±9
Kursk millet	483±11

Voronezh proso, according to these figures, is able to produce nearly 2 pounds of grain with the water required for the production of 1 pound of alfalfa hay.

Kursk millet was also included in the 1913 measurements. Its water requirement was 286±4, or 53 per cent higher than in 1912. Many of the plants were broken by a high wind shortly before harvest, which greatly reduced the grain yield. The mean water requirement, based on grain production, has consequently been omitted.

LEGUMES

The legumes tested at Akron in 1912 included sweet clover, chick-pea, and two strains of Grimm alfalfa, one being a selected strain (Pl. V, figs. 4 and 5) developed by Mr. A. C. Dillman. Both the alfalfa and the sweet clover showed a marked reduction in water requirement compared with the results obtained in 1911. Three cuttings were made in the case of each crop, but the plants were not mature at the time

the last cutting was made. The following values (Table XVIII) were obtained for the water-requirement ratio:

TABLE XVIII.—*Summary of water-requirement measurements of legumes at Akron, Colo., in 1912*

Crop.	Cutting.			
	First.	Second.	Third.	Combined.
Alfalfa, Grimm	592±13	790±10	506±5	659±6
Alfalfa, Grimm, A. D. I. selection	600±17	853±13	421±10	657±11
Clover, sweet	547±12	677±14	598±18	638±4
Chick-pea	.....	.....	.....	510±14

The two alfalfas and the sweet clover were planted on the same day, and the crops in each instance were all cut on the same day, so that the results in the Table XVIII are comparable. The A. D. I. strain of Grimm alfalfa gave a slightly higher ratio than the unselected Grimm during the second period, but lower during the third period, when it made a better growth. (See "Dry matter," column 3, Table XIX.) Sweet clover, as in 1911, proved somewhat more efficient than alfalfa during the first and second periods. During the third period sweet clover was less efficient than alfalfa.

The chick-pea proved the most efficient of the legumes tested. Its growth period does not coincide with that of the other legumes, but approximates a combination of the first and second periods. (See Table XIX.) It thus appears to be distinctly more efficient in the use of water than either alfalfa or sweet clover. Chick-pea has, however, a relatively high water requirement compared with the small grain crops, which is in accord with Leather's measurements (Leather, 1910, p. 156).

TABLE XIX.—*Water requirement of different legumes at Akron, Colo., in 1912*

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Alfalfa, Grimm, S. P. I. 25695 (*Medicago sativa*), first crop, May 23 to July 26...	127	110.3	.....	60.3	.....	.....	548
	128	118.5	.....	77.3	.....	.....	652
	129	141.4	.....	86.1	.....	.....	609
	130	124.2	.....	74.8	.....	.....	602
	131	112.4	.....	59.9	.....	.....	533
	132	156.8	.....	95.0	.....	.....	606
Mean	.....	.....	.....	.....	.....	.....	592±13
Alfalfa, Grimm, second crop, July 26 to Sept. 6	127	141.2	.....	112.4	.....	.....	796
	128	112.5	.....	97.0	.....	.....	862
	129	129.1	.....	100.8	.....	.....	781
	130	125.0	.....	98.8	.....	.....	790
	131	131.2	.....	100.1	.....	.....	763
	132	168.3	.....	125.8	.....	.....	748
Mean	.....	.....	.....	.....	.....	.....	700±10

TABLE XIX.—*Water requirement of different legumes at Akron, Colo., in 1912*—Contd.

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Alfalfa, Grimm, third crop, Sept. 6 to Nov. 4	127	68. 2	.....	36. 5	.....	.....	536
	128	65. 7	.....	32. 4	.....	.....	493
	129	59. 9	.....	29. 6	.....	.....	494
	130	51. 9	.....	26. 9	.....	.....	518
	131	48. 2	.....	24. 4	.....	.....	506
	132	60. 2	.....	29. 3	.....	.....	487
Mean							506±5
Alfalfa, Grimm, combined crop, May 23 to Nov. 4	127	319. 7	.....	209. 2	.....	.....	654
	128	296. 7	.....	206. 7	.....	.....	697
	129	330. 4	.....	216. 5	.....	.....	656
	130	301. 1	.....	200. 5	.....	.....	666
	131	291. 8	.....	184. 4	.....	.....	632
	132	385. 3	.....	250. 1	.....	.....	649
Mean							659±6
Alfalfa, Grimm A. D. I. E–23–20–52, first crop, May 24 to July 26	133	131. 6	.....	89. 6	.....	.....	680
	134	121. 4	.....	73. 9	.....	.....	609
	135	141. 0	.....	82. 4	.....	.....	584
	136	140. 6	.....	72. 5	.....	.....	516
	137	141. 4	.....	79. 8	.....	.....	564
	138	118. 0	.....	76. 6	.....	.....	649
Mean							600±17
Alfalfa, Grimm, second crop, July 26 to Sept. 6	133	131. 7	.....	118. 8	.....	.....	902
	134	110. 0	.....	93. 0	.....	.....	845
	135	138. 6	.....	112. 4	.....	.....	810
	136	133. 3	.....	115. 8	.....	.....	870
	137	135. 2	.....	108. 1	.....	.....	800
	138	120. 2	.....	107. 4	.....	.....	893
Mean							853±13
Alfalfa, Grimm, third crop, Sept. 6 to Nov. 4	133	73. 7	.....	35. 4	.....	.....	479
	134	64. 7	.....	28. 1	.....	.....	434
	135	80. 7	.....	32. 4	.....	.....	401
	136	82. 0	.....	32. 0	.....	.....	390
	137	83. 8	.....	36. 1	.....	.....	431
	138	79. 5	.....	31. 1	.....	.....	391
Mean							421±10
Alfalfa, Grimm, combined crop, May 24 to Nov. 4	133	337. 0	.....	243. 8	.....	.....	724
	134	296. 1	.....	195. 0	.....	.....	658
	135	359. 6	.....	227. 2	.....	.....	632
	136	355. 9	.....	220. 3	.....	.....	620
	137	360. 4	.....	224. 4	.....	.....	627
	138	317. 7	.....	215. 1	.....	.....	678
Mean							657±11
Clover, sweet, S. P. I. 21216(*Melilotus alba*), first crop, May 23 to July 26	121	108. 2	.....	61. 4	.....	.....	568
	122	61. 5	.....	36. 8	.....	.....	598
	123	64. 4	.....	33. 6	.....	.....	522
	124	69. 5	.....	37. 7	.....	.....	542
	125	137. 0	.....	68. 9	.....	.....	503
Mean							547±12

TABLE XIX.—*Water requirement of different legumes at Akron, Colo., in 1912*—Contd.

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1912.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Clover, sweet, second crop, July 26 to Sept. 6	121	155.3	.....	111.6	.....	.....	719
	122	130.5	.....	87.5	.....	.....	670
	123	103.8	.....	74.7	.....	.....	720
	124	143.9	.....	94.5	.....	.....	657
	125	149.0	.....	92.3	.....	.....	620
Mean		.....	.....	.....	.....	.....	677±14
Clover, sweet, third crop, Sept. 6 to Nov. 7	121	23.9	.....	12.5	.....	.....	522
	122	19.4	.....	12.0	.....	.....	619
	123	15.3	.....	9.3	.....	.....	608
	124	14.8	.....	9.5	.....	.....	642
Mean		.....	.....	.....	.....	.....	598±18
Clover, sweet, combined crop, May 23 to Nov. 7	121	287.4	.....	185.5	.....	.....	645
	122	211.4	.....	136.3	.....	.....	645
	123	183.5	.....	117.6	.....	.....	641
	124	228.3	.....	141.7	.....	.....	621
Mean		.....	.....	.....	.....	.....	638±4
Chick-pea, S. P. I. 24322 (*Cicer arietinum*), June 3 to Aug. 30	157	239.9	97.0	138.0	40	1,423	576
	158	275.5	126.3	129.1	46	1,022	469
	159	238.3	85.0	127.1	36	1,495	533
	160	181.1	47.2	96.0	26	2,032	530
	161	272.0	120.1	129.4	44	1,077	476
	162	227.0	104.5	108.6	46	1,039	478
Mean		.....	.....	.....	.....	1,348±114	510±14

A number of different legumes were included in the 1913 water-requirement measurements at Akron (Table XX). On the basis of dry matter produced, the results obtained are as follows:

Kind of legume	Water requirement
Cowpea	571±3
Soy bean	672±9
Navy bean	682±4
Peruvian alfalfa	651±12
Hairy vetch	690±8
Horse bean, S. P. I. 25645	772±11
Mexican bean	773±8
Canada pea	775±5
Horse bean, S. P. I. 15429	780±19
Red clover	789±9
Crimson clover	805±8
Wild soy bean	815±25
Grimm alfalfa	834±8
Purple vetch	935±9

Cowpea (Pl. VI, fig. 1) was the most efficient of the cultivated legumes. The least efficient was purple vetch. The water requirement of the first crop of hairy vetch (Pl. VI, fig. 2) was 9 per cent less than for purple vetch, the period of growth being the same for both varieties. Some of the pots of hairy vetch produced a good second growth, but none of the pots of purple vetch were able to survive the first cutting. The water requirement of the combined crops of hairy vetch was 690±8, or about three-fourths that of purple vetch. Of the soy beans (Pl. VI, fig. 3) the wild required 21 per cent more water than the cultivated variety, which in turn required 18 per cent more water than cowpea. (See Table XX.)

TABLE XX.—*Water requirement of legumes at Akron, Colo., in 1913*

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1913.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Alfalfa, Grimm, E–23–20–52 (*Medicago sativa*), first crop, June 7 to July 18	55	120.8	.....	104.1	.....	.....	862
	56	110.0	.....	91.7	.....	.....	834
	57	99.0	.....	72.3	.....	.....	730
	58	114.8	.....	89.8	.....	.....	782
	59	114.9	.....	94.0	.....	.....	818
	60	110.5	.....	86.9	.....	.....	786
	61	98.0	.....	80.8	.....	.....	824
	62	104.5	.....	79.5	.....	.....	760
	63	104.7	.....	87.1	.....	.....	832
	64	97.5	.....	84.8	.....	.....	870
	65	96.5	.....	73.7	.....	.....	764
	66	117.6	.....	92.1	.....	.....	783
Mean	.....	.....	.....	.....	.....	.....	804±9
Alfalfa, Grimm, second crop, July 18 to Aug. 26	55	115.8	.....	106.7	.....	.....	922
	56	106.4	.....	95.7	.....	.....	900
	57	108.7	.....	89.7	.....	.....	825
	58	114.8	.....	96.3	.....	.....	838
	59	101.1	.....	95.1	.....	.....	940
	60	95.8	.....	84.4	.....	.....	881
	61	102.7	.....	88.9	.....	.....	866
	62	104.1	.....	91.9	.....	.....	882
	63	114.0	.....	98.2	.....	.....	861
	64	85.7	.....	80.3	.....	.....	937
	65	98.9	.....	86.1	.....	.....	871
	66	109.6	.....	89.1	.....	.....	812
Mean	.....	.....	.....	.....	.....	.....	878±8
Alfalfa, Grimm, third crop, Aug. 26 to Oct. 23	55	89.3	.....	77.5	.....	.....	868
	56	82.2	.....	70.4	.....	.....	856
	57	73.8	.....	55.5	.....	.....	752
	58	76.6	.....	59.1	.....	.....	771
	59	81.9	.....	70.4	.....	.....	860
	60	79.3	.....	63.0	.....	.....	794
	61	85.5	.....	63.3	.....	.....	740
	62	76.5	.....	60.4	.....	.....	790
	63	88.9	.....	76.8	.....	.....	864
	64	66.9	.....	56.4	.....	.....	843
	65	71.3	.....	61.7	.....	.....	866
	66	87.6	.....	70.7	.....	.....	807
Mean	.....	.....	.....	.....	.....	.....	818±11

TABLE XX.—*Water requirement of legumes at Akron, Colo., in 1913*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1913.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Alfalfa, Grimm, combined crop, June 5 to Oct. 23	55	325.9	.....	288.3	.....	.....	886
	56	298.6	.....	257.8	.....	.....	864
	57	281.5	.....	217.5	.....	.....	773
	58	306.2	.....	245.2	.....	.....	801
	59	297.9	.....	259.5	.....	.....	871
	60	285.6	.....	234.3	.....	.....	821
	61	286.2	.....	233.0	.....	.....	814
	62	285.1	.....	231.8	.....	.....	813
	63	307.6	.....	262.1	.....	.....	853
	64	250.1	.....	221.5	.....	.....	886
	65	266.7	.....	221.5	.....	.....	831
	66	314.8	.....	251.9	.....	.....	800
Mean		.....	.....	.....	.....	.....	834±8
Alfalfa, Peruvian, S. P. I. 30203 (*Medicago sativa*), first crop, June 7 to July 19	97	41.5	.....	26.3	.....	.....	634
	98	70.3	.....	45.5	.....	.....	648
	99	66.0	.....	38.9	.....	.....	590
	100	45.0	.....	27.3	.....	.....	606
	101	78.8	.....	54.8	.....	.....	695
	102	87.9	.....	64.4	.....	.....	733
Mean		.....	.....	.....	.....	.....	651±16
Alfalfa, Peruvian, second crop, July 19 to Aug. 26	97	62.0	.....	42.5	.....	.....	685
	98	104.5	.....	73.3	.....	.....	701
	99	91.4	.....	63.9	.....	.....	699
	100	69.9	.....	47.0	.....	.....	672
	101	88.4	.....	69.5	.....	.....	786
	102	102.3	.....	82.5	.....	.....	806
Mean		.....	.....	.....	.....	.....	725±18
Alfalfa, Peruvian, third crop, Aug. 26 to Oct. 23	97	63.6	.....	39.9	.....	.....	627
	98	99.2	.....	55.5	.....	.....	559
	99	85.0	.....	49.9	.....	.....	587
	100	74.5	.....	42.6	.....	.....	572
	101	(a)	.....	58.8	.....	.....	.....
	102	108.9	.....	69.3	.....	.....	636
Mean		.....	.....	.....	.....	.....	596±12
Alfalfa, Peruvian, combined crop, June 7 to Oct. 23	97	167.1	.....	108.7	.....	.....	650
	98	274.0	.....	174.3	.....	.....	636
	99	242.4	.....	152.7	.....	.....	630
	100	189.4	.....	116.9	.....	.....	618
	102	299.1	.....	216.2	.....	.....	723
Mean		.....	.....	.....	.....	.....	651±12
Clover, red, S. P. I. 34869 (*Trifolium repens*), first crop, June 5 to July 19	103	99.7	.....	73.1	.....	.....	733
	104	71.0	.....	56.0	.....	.....	789
	105	89.6	.....	60.3	.....	.....	673
	106	92.6	.....	63.6	.....	.....	687
	107	116.3	.....	82.0	.....	.....	705
	108	112.8	.....	81.1	.....	.....	719
Mean		.....	.....	.....	.....	.....	718±11

[a] Missing.

TABLE XX.—*Water requirement of legumes at Akron, Colo., in 1913*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1913.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Clover, red, second crop, July 19 to Aug. 26	103	62.7	.....	53.9	.....	.....	860
	104	66.0	.....	53.0	.....	.....	803
	105	39.5	.....	36.0	.....	.....	911
	106	62.8	.....	53.9	.....	.....	858
	107	52.5	.....	45.7	.....	.....	870
	108	46.6	.....	44.4	.....	.....	953
Mean	.....	.....	.....	.....	.....	.....	876±14
Clover, red, third crop, Aug. 26 to Oct. 22	103	13.6	.....	17.0	.....	.....	1,250
	104	18.3	.....	17.4	.....	.....	952
	105	9.7	.....	10.7	.....	.....	1,103
	106	8.5	.....	9.0	.....	.....	1,058
	107	37.7	.....	26.0	.....	.....	689
	108	10.4	.....	12.9	.....	.....	1,040
Mean	.....	.....	.....	.....	.....	.....	1,015±49
Clover, red, combined crop, June 5 to Oct. 22	103	176.0	.....	144.0	.....	.....	818
	104	155.3	.....	126.4	.....	.....	814
	105	138.8	.....	107.0	.....	.....	771
	106	163.9	.....	126.5	.....	.....	772
	107	206.5	.....	153.7	.....	.....	745
	108	169.8	.....	138.4	.....	.....	815
Mean	.....	.....	.....	.....	.....	.....	789±9
Clover, crimson, S. P. I. 33742 (*Trifolium incarnatum*), June 5 to Aug. 26	109	191.3	.....	160.8	.....	.....	841
	110	169.2	.....	131.0	.....	.....	774
	111	186.2	.....	150.5	.....	.....	809
	112	146.7	.....	118.1	.....	.....	806
	113	109.8	.....	84.9	.....	.....	773
	114	110.2	.....	91.0	.....	.....	825
Mean	.....	.....	.....	.....	.....	.....	805±8
Vetch, hairy, S. P. I. 34298 (*Vicia villosa*), first crop, May 29 to July 18	181	119.4	.....	93.3	.....	.....	781
	182	97.8	.....	76.9	.....	.....	786
	183	98.1	.....	95.9	.....	.....	977
	184	114.5	.....	99.6	.....	.....	870
	185	121.9	.....	98.9	.....	.....	811
	186	120.2	.....	106.9	.....	.....	890
Mean	.....	.....	.....	.....	.....	.....	853±23
Vetch, hairy, second crop, July 18 to Oct. 22	181	114.8	.....	61.7	.....	.....	537
	184	148.4	.....	80.1	.....	.....	540
	185	155.7	.....	92.6	.....	.....	595
	186	131.7	.....	74.7	.....	.....	567
Mean	.....	.....	.....	.....	.....	.....	560±10
Vetch, hairy, combined crop, June 5 to Oct. 22	181	234.2	.....	155.0	.....	.....	662
	184	262.9	.....	179.7	.....	.....	684
	185	277.6	.....	191.5	.....	.....	690
	186	251.9	.....	181.6	.....	.....	722
Mean	.....	.....	.....	.....	.....	.....	690±8

TABLE XX.—*Water requirement of legumes at Akron, Colo., in 1913*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1913.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Vetch, purple, S. P. I. 18131 (*Vicia atropurpurea*), May 29 to July 18	187	117.5	.....	113.7	.....	.....	967
	188	118.5	.....	108.8	.....	.....	919
	189	128.9	.....	119.8	.....	.....	930
	190	113.8	.....	106.0	.....	.....	932
	191	102.4	.....	99.6	.....	.....	972
	192	115.2	.....	102.3	.....	.....	888
Mean						.....	935±9
Pea, Canada field, S. P. I. 30134 (*Pisum sativum*), June 3 to Aug. 4	241	154.0	64.7	117.9	42	1,822	766
	242	137.2	50.3	104.5	37	2,078	762
	243	136.3	46.6	103.8	34	2,228	762
	244	134.2	51.4	103.0	38	2,202	768
	245	120.0	32.8	96.0	27	2,921	800
	246	68.4	20.2	54.1	29	2,679	791
Mean						2,322±121	775±5
Bean, Mexican (*Phaseolus vulgaris*), May 28 to Sept. 1	229	282.4	120.8	213.9	43	1,769	757
	230	269.0	117.0	205.1	43	1,753	763
	231	260.7	108.9	191.0	42	1,755	733
	232	232.8	100.7	182.8	43	1,815	786
	233	276.2	111.9	218.4	40	1,952	791
	234	245.3	86.7	198.0	35	2,285	807
Mean						1,888±62	773±8
Bean, navy (*Phaseolus vulgaris*), May 28 to Aug. 21	223	220.3	101.2	144.6	46	1,429	656
	224	204.0	87.9	142.5	43	1,621	699
	225	224.5	95.6	153.3	43	1,604	683
	226	168.1	68.1	116.8	40	1,715	694
	227	227.9	90.5	153.3	40	1,693	673
	228	186.1	70.4	127.6	38	1,813	686
Mean						1,646±36	682±4
Bean, soy, S. P. I. 21755 (*Glycine hispida*), June 1 to Aug. 26	193	156.2	51.8	100.3	33	1,938	642
	194	144.7	51.9	91.4	36	1,760	631
	195	150.4	49.9	103.6	33	2,078	689
	196	158.2	48.5	110.3	31	2,275	698
	197	154.1	48.2	103.9	31	2,154	674
	198	160.0	52.9	111.7	33	2,110	698
Mean						2,053±51	672±9
Bean, soy, wild, S. P. I. 25138 (*Glycine soja*), June 12 to Sept. 14	199	255.9	.....	238.8	.....	.....	934
	200	305.0	.....	262.1	.....	.....	859
	201	346.1	.....	260.6	.....	.....	754
	202	303.0	.....	257.7	.....	.....	850
	203	374.9	.....	262.8	.....	.....	701
	204	329.7	.....	260.5	.....	.....	790
Mean						.....	815±25

TABLE XX.—*Water requirement of legumes at Akron, Colo., in 1913*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1913.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Cowpea, S. P. I. 29282 (*Vigna sinensis*), June 17 to Aug. 26...	151	207.0	69.9	123.1	34	1,761	595
	152	202.6	71.4	115.6	35	1,620	571
	153	215.7	80.1	121.7	37	1,519	564
	154	209.2	77.7	116.1	37	1,495	555
	155	210.6	75.0	120.0	36	1,600	570
	156	202.7	79.5	116.1	39	1,460	573
Mean						1,576±32	571±3
Bean, horse, S. P. I. 15429 (*Vicia faba*), June 12 to July 19...	169	38.3	.....	31.0	.....	.....	809
	170	53.7	.....	38.4	.....	.....	715
	171	44.6	.....	33.4	.....	.....	749
	172	30.0	.....	21.7	.....	.....	723
	173	10.0	.....	8.7	.....	.....	870
	174	17.4	.....	14.1	.....	.....	811
Mean							780±19
Bean, horse, S. P. I. 25645 (*Vicia faba*), June 17 to July 9....	175	9.4	.....	7.1	.....	.....	755
	176	40.9	.....	34.0	.....	.....	831
	177	29.4	.....	21.8	.....	.....	742
	178	53.6	.....	41.5	.....	.....	774
	179	18.5	.....	13.5	.....	.....	730
	180	10.4	.....	8.3	.....	.....	798
Mean							772±11

Neither variety of horse bean did well. The growth was fairly good during the early period, but during the warm days of July the plants wilted down badly, despite an ample water supply, and had to be harvested before they had reached maturity. The water requirement, notwithstanding this, is no higher than that of many of the other legumes, and compares favorably in this respect with hairy and with purple vetch. The navy bean, although not as efficient as the cowpea and the soy bean, is more efficient than the Mexican bean, which required 13 per cent more water. The Canada field pea and the Mexican bean were equally efficient.[1]

Crimson clover, on the basis of the combined crop, required practically the same quantity of water as red clover. Crimson clover produced only one crop and grew slowly throughout the period, although in total production it was practically the equal of red clover. The water requirement of red clover is slightly below that of Grimm alfalfa, while Peruvian alfalfa required only 78 per cent as much water as Grimm for the pro-

[1] Peas and beans were included by Lawes (1850, p. 54) in his experiments at Rothamstead, England. His measurements show beans to be slightly more efficient than peas. No other measurements of peas and beans have been made, so far as the writers are aware.

duction of a pound of dry matter. The total dry matter produced by Peruvian was, however, much less. The water requirement for each of the several cuttings made of these crops is shown in Table XXI.

TABLE XXI.—*Water requirement of different cuttings of legumes*

Crop.	First cutting.	Second cutting.	Third cutting.	Combined cutting.
Alfalfa, Peruvian	651±16	725±18	596±12	651±12
Clover, red	718±11	876±14	1,015±49	789±9
Alfalfa, Grimm	804±9	878±8	818±11	834±8
Vetch, hairy	853±23	560±10	.....	690±8

Taking the water requirement of the first cutting in each instance as a basis of comparison, the second crop of Grimm alfalfa required 8 per cent more water, Peruvian alfalfa 11 per cent more, red clover 22 per cent more, while hairy vetch required 34 per cent less. On the same basis the third cutting of Grimm alfalfa required 2 per cent more water than the first, 8 per cent more for Peruvian, and red clover 41 per cent more. Comparing the combined cuttings, Peruvian alfalfa and hairy vetch required 18 per cent less water than Grimm, and red clover 5 per cent less. Grimm alfalfa was the only one of the lupines grown at Akron during both 1912 and 1913. Its water requirement in 1912 was 659±16; in 1913, 834±8, an increase of 27 per cent.

ALFALFA, SUDAN GRASS, AND MILLET GROWN DURING THE LATE SUMMER AND AUTUMN AT AKRON, COLO., IN 1912

In Table XXII are given the results of water-requirement measurements based on the total dry matter produced by crops grown during the late summer and autumn and planted in pots which had already produced a crop earlier in the season. The soil was not changed, and no additional fertilizer was added. These forage crops can not be said to have been grown out of season, except in that the plantings were made too late in the season to permit the plants to reach their full development before harvesting.

TABLE XXII.—*Water requirement of alfalfa, Sudan grass, and millet grown during the late summer and autumn at Akron, Colo., in 1912, without additional fertilizer.*

Crop.	Pot No.	Dry matter.	Water.	Water requirement based on dry matter.
1912.		*Grams.*	*Kilos.*	
Alfalfa, Grimm, E-23-20-52 (*Medicago sativa*), Aug. 7 to Nov. 6, following Kharkov wheat	37	40.9	40.4	988
	39	43.1	37.3	866
	40	33.2	31.7	955
	41	32.0	30.2	944
	42	34.3	34.9	1,017
Mean				954±10

TABLE XXII.—*Water requirement of alfalfa, Sudan grass, and millet grown during the late summer and autumn at Akron, Colo., in 1912, without additional fertilizer*—Continued

Crop.	Pot No.	Dry matter.	Water.	Water requirement based on dry matter.
		Grams.	*Kilos.*	
Alfalfa, Grimm, following Turkey wheat	43	40.4	34.8	862
	44	45.3	40.5	894
	45	21.0	21.3	1,013
	46	25.6	23.0	899
	47	30.7	26.9	876
	48	33.0	30.7	930
Mean				912±15
Alfalfa, Grimm, Sept. 3 to Nov. 7, following Bluestem wheat	31	14.7	7.5	510
	32	16.8	8.5	506
	33	18.1	8.3	458
	34	19.6	8.1	413
	35	13.8	6.1	442
	36	12.9	6.6	511
Mean				473±13
Alfalfa, Grimm, following unfertilized Kubanka wheat	7	14.7	9.3	628
	8	19.8	10.8	545
	9	15.0	9.1	606
	10	16.4	9.5	579
	12	16.7	9.5	569
Mean				585±11
Alfalfa, Grimm, Sept. 9 to Nov. 4, following *Grindelia squarrosa*	163	12.9	7.5	581
	164	11.8	6.4	542
Mean				564±16
Alfalfa, yellow flowered (*Medicago falcata*), Sept. 3 to Nov. 7, following Kubanka wheat	4	10.0	4.6	460
	5	10.8	5.1	472
	6	16.5	8.3	503
Mean				478±10
Sudan grass, S. P. I. 25017 (*Andropogon sorghum aethiopicus*), Sept. 3 to Oct. 1, following spring Ghirka wheat	55	11.5	3.0	261
	56	18.1	5.4	298
	57	10.5	3.1	295
	58	19.5	5.8	297
	59	16.6	5.7	343
	60	9.6	3.4	354
Mean				308±10
Proso, Black Voronezh, S. D. 331 (*Panicum miliaceum*), Aug. 22 to Sept. 28, following Beldi barley	97	20.0	4.4	220
	98	25.0	5.8	232
	99	19.5	3.7	190
	101	15.5	4.0	258
	102	20.0	4.6	230
Mean				226±7

TABLE XXII.—*Water requirement of alfalfa, Sudan grass, and millet grown during the late summer and autumn at Akron, Colo., in 1912, without additional fertilizer*—Continued

Crop.	Pot No.	Dry matter.	Water.	Water requirement based on dry matter.
		Grams.	*Kilos.*	
Millet, Turkestan, S. P. I. 20694 (*Chaetochloa italica*), Aug. 22 to Oct. 1, following White Hull-less barley	103	27.0	7.8	289
	104	28.3	8.5	300
	105	30.7	8.4	273
	106	30.4	8.9	293
	107	33.5	9.2	274
	108	35.1	11.7	333
Mean				294±6
Millet, Kursk, S. P. I. 30029 (*Chaetochloa italica*), Sept. 3 to Oct. 3, following spring rye	115	9.3	1.8	194
	116	12.4	2.5	202
	117	19.3	4.0	207
	118	24.2	3.9	161
	119	23.5	3.1	132
	120	12.3	1.8	146
Mean				173±10
Millet, Kursk, S. P. I. 34771, Sept. 3 to Oct. 1, following emmer	61	22.2	3.7	167
	62	18.4	2.9	158
	63	23.4	3.7	158
	64	21.6	3.8	176
	65	24.7	3.7	150
	66	24.5	3.8	155
Mean				161±3

The effect of fertilizer added to the previous crop on the water requirement of the next crop is shown in the following measurements:

Crop	Water requirement
Alfalfa, following fertilized Bluestem wheat	473±13
Alfalfa, following unfertilized Kubanka wheat	585±11
Alfalfa, following fertilized Grindelia, following unfertilized Bluestem wheat	564±16

The water requirement of alfalfa following unfertilized Kubanka wheat was higher than that following the fertilized Bluestem, and although this difference is not very marked (19±3 per cent) when the probable error is considered, it is sufficient to show a slight effect of two consecutive crops without fertilizer in increasing the water requirement. Two pots of alfalfa following *Grindelia squarrosa* had a water requirement equal to that of the unfertilized set. Grindelia was started in pots which had already produced a crop of Marvel Bluestem wheat in 1911 without fertilizer. Therefore alfalfa was the third crop from the same soil mass, and although the pots growing Grindelia were fertilized, the succeeding alfalfa crop gave a water requirement in accord with that following unfertilized wheat.

The effect of time of planting is shown in the following determinations of the water requirement of alfalfa:

Crop	Water requirement
Alfalfa, grown August 7 to November 6, following Kharkov wheat	954±16
Alfalfa, grown August 7 to November 6, following Turkey wheat	912±15
Alfalfa, grown September 3 to November 7, following Bluestem wheat	473±13

Alfalfa planted during the dry, hot days of August required almost twice as much water for the production of a unit of dry matter as it did when planted in September.

The seven varieties and strains given in Table XXIII were included in these late-season experiments and were grown under comparable conditions. The water requirement of Grimm alfalfa, Sudan grass, and Kursk millet was also determined in midsummer (see column 3), so that it is possible to reduce the water-requirement measurement of the other late-season crops to a midsummer basis. The seasonal water requirement of Grimm alfalfa was 39 per cent higher than that of the late-season crop. Assuming this ratio to hold for the yellow-flowered alfalfa, the seasonal water requirement of the latter would be 865±18. The midsummer water requirement of Sudan grass and of Kursk millet (S. P. I. 34771) was in each instance 16 per cent above the late-season crop, and this ratio has been used in computing the other millets to a midsummer basis. The computed values (*a*) are given in the last column of Table XXIII.

TABLE XXIII.—*Water requirement of late-season crops*

Variety.	Water requirement.	
	Late-season crop.	Midsummer crop.
Alfalfa, yellow-flowered	478±10	[a] 865±18
Alfalfa, Grimm	473±13	657±11
Sudan grass	308±10	359±2
Millet, Turkestan	294±6	[a] 341±7
Proso, Black Voronezh	226±7	[a] 262±8
Millet, Kursk, S. P. I. 30029	173±10	[a] 201±12
Millet, Kursk, S. P. I. 34771	161±3	187±2

[a] Computed.

Of these seven varieties the yellow-flowered alfalfa (*Medicago falcata*) gave a water requirement in practical accord with the Grimm selection grown during the same period. Kursk millet (S. P. I. 34771) gave the lowest water requirement and proved to be decidedly more efficient than Black Voronezh proso and Turkestan millet. Sudan grass required 91 per cent more water than the Kursk millet, which is in exact accord with the results obtained for these two crops from determinations based on a whole season's growth.

CUCURBITS

On account of the large space required by crops which produce vines, the cucurbits were grown outside the inclosure at Akron in 1913. The reduction in water requirement produced by the inclosure amounted in the case of wheat, alfalfa, and cocklebur to approximately 20 per cent. These ratios for the cucurbits should therefore be reduced by this amount in comparing them with crops grown inside the shelter. The observed water requirement outside and the computed water requirement within the inclosure, both based on dry matter, follow:

Crop	Outside	Inside
Watermelon	750±19	600±15
Cantaloupe	778±34	621±27
Cucumber	891±14	713±11
Squash	936±10	748±8
Pumpkin	1,043±21	834±17

The cucumber, cantaloupe (Pl. VI, fig. 4), and watermelon did well in the pots. Squash and pumpkin produced very little fruit (Table XXIV), and the growth of vines was not normal. Watermelon and muskmelon proved to be the most efficient of the cucurbits. Pumpkin, the highest of the cucurbits in water requirement, is about the equal of alfalfa in efficiency.

TABLE XXIV.—*Water requirement of cucurbits at Akron, Colo., in 1913*

Plant and period of growth.	Pot No.	Dry matter.	Dry matter in fruit.	Water.	Fruit.	Water requirement based on—	
						Fruit.	Dry matter.
1913.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Squash, Hubbard (*Cucurbita maxima*), June 3 to Sept. 13	337	272.0	46.1	260.7	17	5,658	959
	338	280.9	85.6	252.1	30	2,945	898
	339	244.3	33.4	224.2	14	6,715	918
	340	247.3	18.4	242.9	7	.....	982
	341	267.9	68.6	244.9	26	3,570	914
	342	258.9	7.3	244.7	3	.....	946
Mean						4,720±690	936±10
Pumpkin, common field (*Cucurbita pepo*), June 3 to Sept. 13	343	205.9	.....	215.6	.....	.....	1,047
	344	222.4	5.9	221.0	3	.....	994
	345	228.8	45.9	211.6	20	.....	925
	346	225.4	2.8	243.2	1	.....	1,078
	347	190.5	.....	215.2	.....	.....	1,130
	348	179.7	2.8	194.6	2	.....	1,083
Mean						.....	1,043±21
Cucumber, Boston pickling (*Cucumis sativus*), June 14 to Sept. 1	319	171.1	101.5	154.8	59	1,525	905
	320	185.6	100.0	167.6	54	1,676	903
	321	185.7	96.3	158.2	42	1,642	852
	322	182.5	112.4	152.9	62	1,360	838
	323	175.8	83.7	171.1	48	2,045	974
	324	174.8	107.4	152.3	61	1,417	872
Mean						1,611±67	891±14

TABLE XXIV.—*Water requirement of cucurbits at Akron, Colo., in 1913*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Dry matter in fruit.	Water.	Fruit.	Water requirement based on—	
						Fruit.	Dry matter.
1913.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Cantaloupe, Rocky Ford (*Cucumis melo*), June 14 to Sept. 13..	325	314.7	198.9	201.7	63	1,014	641
	326	285.3	155.2	214.4	55	1,382	752
	327	211.4	92.5	210.5	44	2,276	996
	328	264.9	132.5	218.1	50	1,646	824
	329	272.8	160.6	197.3	59	1,228	723
	330	305.6	162.4	222.8	53	1,371	729
Mean.....						1,486±120	778±34
Watermelon, Rocky Ford (*Citrullus vulgaris*), June 14 to Sept. 13.....	331	301.8	193.2	238.2	64	1,233	790
	332	318.5	216.8	215.5	68	995	677
	333	334.5	225.9	241.4	67	1,069	722
	334	314.2	209.1	232.8	66	1,112	740
	335	267.9	159.4	232.1	59	1,457	866
	336	320.8	224.2	226.5	70	1,010	706
Mean.....						1,146±49	750±19

On the basis of the production of fruit the watermelon has proven to be exceptionally efficient. The water requirement, calculated on the basis of the dry matter in the melons and reduced to inclosure conditions, was 915±39. The green fruit contained 95 per cent of water. The water requirement on a green basis would therefore be 46.

RAPE, TURNIP, CABBAGE, AND POTATO

Rape, turnip, cabbage, and two varieties of potato, the Irish Cobbler and the McCormick (Pl. VI, fig. 6), were included in the 1913 measurements (Table XXV). The water requirement, based on dry matter, was as follows:

Crop	Water requirement
Cabbage	539±7
Turnip	639±31
Potato:	
Irish Cobbler	659±15
McCormick	717±11
Rape	743±7

Cabbage and turnip are seen to have a lower water requirement than potato and to rank in efficiency with oats. Of the potato varieties the Irish Cobbler was the more efficient and produced the most tubers.

The McCormick, a late-maturing variety, produced fine vines but practically no tubers. The water requirement of rape was practically the same as that of turnip during the same period of growth, but the second crop, although not a heavy one, had a water requirement so much higher than the first that the combined crop is approximately 16 per cent higher than for turnip.

TABLE XXV.—*Water requirement of rape, turnip, cabbage, and potato at Akron, Colo., in 1913*

Plant and period of growth.	Pot No.	Dry matter.	Tubers or roots.	Water.	Tubers or roots.	Water requirement based on—	
						Tubers or roots.	Dry matter.
1913.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Rape (*Brassica napus*), June 3 to July 19....	217	171.2	.....	112.1	.....	.....	655
	218	156.3	.....	102.9	.....	.....	658
	219	162.0	.....	102.5	.....	.....	633
	220	140.5	.....	97.4	.....	.....	693
	221	171.5	.....	108.0	.....	.....	630
	222	164.6	.....	110.5	.....	.....	671
Mean.....	.....	.....	.....	.....	.....	.....	657±6
Rape, second crop, July 19 to Sept. 13..	217	44.6	.....	54.3	.....	.....	1,217
	218	68.5	.....	69.2	.....	.....	1,010
	219	58.3	.....	58.0	.....	.....	995
	220	63.8	.....	53.2	.....	.....	834
	221	66.4	.....	62.8	.....	.....	946
	222	69.0	.....	60.9	.....	.....	882
Mean.....	.....	.....	.....	.....	.....	.....	981±35
Rape, combined crop, June 3 to Sept. 13...	217	215.8	.....	166.4	.....	.....	771
	218	224.8	.....	172.1	.....	.....	767
	219	220.3	.....	160.5	.....	.....	729
	220	204.3	.....	150.6	.....	.....	737
	221	237.9	.....	170.8	.....	.....	718
	222	233.6	.....	171.4	.....	.....	734
Mean.....	.....	.....	.....	.....	.....	.....	743±7
Turnip, Purple Top (*Brassica rapa*), May 29 to July 19.....	211	128.9	69.3	78.1	54	1,127	606
	212	128.4	64.6	90.7	50	1,403	706
	213	58.6	17.3	30.6	29	1,767	522
	214	128.5	58.8	69.5	45	1,181	541
	215	73.8	37.6	51.2	51	1,360	694
	216	90.4	29.4	68.9	32	2,341	762
Mean.....	.....	.....	.....	.....	.....	1,530±132	639±31
Cabbage, Early Jersey Wakefield (*Brassica oleracea capitata*), June 3 to Sept. 12...	205	284.5	.....	148.9	.....	.....	523
	206	281.9	.....	160.3	.....	.....	569
	207	359.8	.....	191.5	.....	.....	533
	208	330.7	.....	176.0	.....	.....	532
	209	344.6	.....	175.7	.....	.....	510
	210	313.2	.....	177.0	.....	.....	565
Mean.....	.....	.....	.....	.....	.....	.....	539±7
Potato, Irish Cobbler (*Solanum tuberosum*), June 5 to Sept. 12.....	115	170.2	40.6	120.4	24	2,966	708
	116	203.2	67.8	145.1	33	2,140	714
	117	211.0	81.7	127.0	39	1,555	602
	118	222.3	86.6	149.3	39	1,724	671
	119	218.1	105.3	130.0	48	1,234	596
	120	201.1	52.4	133.3	26	2,542	662
Mean.....	.....	.....	.....	.....	.....	2,027±197	659±15
Potato, McCormick (*Solanum tuberosum*), June 5 to Oct. 4.....	121	215.8	.1	145.5	.....	.....	674
	122	213.7	1.7	154.9	.....	.....	724
	123	215.5	5.0	146.9	2	.....	682
	124	219.3	10.3	164.5	5	.....	750
	125	201.4	.3	154.2	.....	.....	766
	126	206.9	1.9	145.9	1	.....	706
Mean.....	.....	.....	.....	.....	.....	.....	717±11

NATIVE AND INTRODUCED GRASSES AND OTHER NATIVE PLANTS

Two native Colorado plants were included in the 1912 measurements at Akron, *Grindelia squarrosa*, or "gum weed," and *Artemisia frigida*, or "mountain sage" (Pl. II, fig. 3). These plants were carried through the winter in the pots, and only two pots of each set were in good condition in the spring. They both behaved as biennials, forming rosettes in 1911 and flowering profusely in 1912. The data (Table XXVI) given for the period from May 20 to August 26 include much of the stored dry matter of the rosettes and root systems elaborated during an earlier period, and consequently the water requirement is somewhat too low. In order to check this, the data based on the total period of growth, which includes the growth and water consumption in 1911, have also been given. This method of computation increases the water requirement less than 6 per cent.

TABLE XXVI.—*Water requirement of native plants at Akron, Colo., in 1912*

Plant and period of growth.	Pot No.	Dry matter.	Water.	Water requirement based on dry matter.
1912.		*Grams.*	*Kilos.*	
Grindelia squarrosa, May 20 to Aug. 26	163	385.4	172.0	446
	164	367.7	180.0	490
Mean				468±18
Artemisia frigida, May 20 to Aug. 26	167	336.3	153.4	456
	168	293.9	143.9	491
Mean				474±14
TOTAL PERIOD OF GROWTH.				
Grindelia squarrosa, Aug. 18, 1911, to Aug. 26, 1912	163	399.6	182.4	457
	164	381.2	200.5	526
Mean				492±29
Artemisia frigida, Jan. 10, 1911, to Aug. 26, 1912	167	372.8	177.4	476
	168	345.8	183.9	532
Mean				504±24

Although these are typical native plants of the high plains, they required about 20 per cent more water than Kubanka wheat and rank higher in water consumption than any of the cultivated grains except rye and rice.

Grasses produce so slowly that it is somewhat difficult to make satisfactory measurements of their water requirement. The 1913 experiments (Table XXVII) included pure buffalo grass, mixed grama and

buffalo grass, western wheat-grass, brome-grass, and a Siberian wheat-grass. Buffalo grass, brome-grass, and wheat-grass each gave two crops, buffalo and grama mixed gave three cuttings, while western wheat-grass produced but one cutting. The combined crops afford the best basis for the comparison of these grasses. On the basis of the total dry matter produced throughout the season, the water requirement is as follows:

Variety of grass	Water requirement
Buffalo	308±22
Grama and buffalo	389±12
Wheat-grass	705±27
Brome-grass	1,016±26
Western wheat-grass	1,076±29

Brome-grass and western wheat-grass are comparatively very inefficient in the use of water, requiring from 22 to 29 per cent more water than alfalfa. Western wheat-grass made a slow growth. Wheat-grass is more efficient than alfalfa, requiring 15 per cent less water. The short grasses made a wonderful showing, the water requirement of pure buffalo grass being only about one-third that of alfalfa, and that of grama and buffalo mixed, 47 per cent. The water requirement of buffalo grass is only 8 per cent above millet, which is one of the most efficient of the introduced plants. The mixed buffalo and grama grass required 36 per cent more water than Kursk millet. These are the first of the native plants to show any marked efficiency in the use of water, although some of the weeds, as will be seen later, are also highly efficient.

TABLE XXVII.—*Water requirement of native and introduced grasses at Akron, Colo., in 1913*

Plant and period of growth.	Pot No.	Dry matter.	Water.	Water requirement based on dry matter.
1913.		*Grams.*	*Kilos.*	
Grama (*Bouteloua gracilis*) and buffalo grass (*Bulbilis dactyloides*), mixed, first crop, June 3 to July 19	139	50.1	17.4	347
	140	26.9	10.7	398
	141	31.8	12.4	390
	142	21.9	7.5	342
	143	22.1	9.8	443
	144	25.3	8.6	340
Mean				377±11
Grama and buffalo grass, mixed, second crop, July 19 to Aug. 26	139	51.7	18.1	350
	140	35.4	13.2	373
	141	38.8	15.6	402
	142	37.9	14.4	380
	143	41.2	16.1	391
	144	26.1	9.7	371
Mean				395± 7

TABLE XXVII.—*Water requirement of native and introduced grasses at Akron, Colo., in 1913*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Water.	Water requirement based on dry matter.
1913.		*Grams.*	*Kilos.*	
Grama and buffalo grass, mixed, third crop, Aug. 26 to Oct. 20	139	14.3	7.4	517
	140	11.6	6.1	526
	141	11.8	7.0	593
	142	15.9	7.1	446
	143	15.0	7.6	507
	144	18.3	4.3	235
Mean				471±26
Grama and buffalo grass, mixed, combined crops, June 3 to Oct. 20	139	116.1	42.9	369
	140	73.9	30.0	406
	141	82.4	35.0	425
	142	75.7	29.0	383
	143	78.3	33.5	428
	144	69.7	22.6	324
Mean				389±12
Buffalo grass (*Bulbilis, dactyloides*), first crop, June 18 to Aug. 26	145	10.0	3.5	350
	146	32.1	9.0	280
	147	18.9	5.0	264
	148	12.9	3.8	295
	149	13.2	3.8	288
	150	8.1	1.5	185
Mean				277±13
Buffalo grass, second crop, Aug. 26 to Oct. 18	145	3.6	1.5	417
	146	3.1	.8	258
	147	2.3	.7	304
	148	5.2	1.9	365
Mean				336±23
Buffalo-grass, combined crops, June 18 to Oct. 18	145	13.6	5.0	368
	146	35.2	9.8	278
	147	21.2	5.7	269
	148	18.1	5.7	315
Mean				308±17
Brome-grass, S. P. I. 29880 (*Bromus inermis*), first crop, May 23 to July 19	133	64.5	62.8	973
	134	75.2	74.6	992
	135	76.0	70.3	925
	136	60.8	58.6	964
	138	79.5	79.3	998
Mean				970±9
Brome-grass, second crop, July 19 to Oct. 22	133	30.3	28.4	937
	134	46.1	59.3	1,286
	135	36.1	46.6	1,290
	136	26.9	21.8	810
	138	40.1	47.0	1,172
Mean				1,099±76

TABLE XXVII.—*Water requirement of native and introduced grasses at Akron, Colo., in 1913*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Water.	Water requirement based on dry matter.
		Grams.	*Kilos.*	
1913.				
Brome-grass, combined crops, May 23 to Oct. 22.	133	94.8	91.2	962
	134	121.3	133.9	1,104
	135	112.1	116.9	1,043
	136	87.7	80.4	916
	138	119.6	126.3	1,056
Mean				1,016±26
Wheat-grass (*Agropyron cristatum*), S. P. I. 19537, first crop, June 5 to July 19	67	25.9	19.2	741
	68	33.6	22.4	667
	69	24.3	16.1	663
	70	23.0	19.3	839
	71	17.1	13.8	808
	72	34.0	25.8	759
Mean				746±21
Wheat-grass, second crop, July 19 to Oct. 22	67	49.0	37.9	773
	68	77.0	45.1	585
	69	53.6	29.3	547
	70	58.0	40.2	693
	71	46.1	34.8	755
	72	66.2	51.3	775
Mean				688±31
Wheat-grass, combined crop, June 5 to Oct. 22	67	74.9	57.1	762
	68	110.6	67.5	610
	69	77.9	45.4	583
	70	81.0	59.5	735
	71	63.2	48.6	769
	72	100.2	77.1	769
Mean				705±27
Wheat-grass, western (*Agropyron Smithii*), June 17 to Oct. 22	235	37.7	45.0	1,193
	236	41.4	43.1	1,040
	237	18.3	15.2	831
	238	21.1	23.0	1,087
	239	25.6	28.9	1,128
	240	16.7	19.7	1,179
Mean				1,076±29

WEEDS

A number of weeds were grown in pots at Akron in 1913, in order to determine their water requirement. (Table XXVIII.) Most of these were planted late in the season, after the crops of grain had been removed. Pigweed and the annual sunflower were, however, started at the beginning of the season. Three crops of pigweed were produced, the water requirement for the first, second, third, and combined cuttings, based on dry matter, being 325±10, 326±4, 278±7, and 320±7, respectively.

Sunflower, which made an excellent growth, gave a water requirement of 705±8. Sunflower thus requires almost three times as much water as pigweed and 86 per cent as much water as alfalfa.

A comparison of the water requirement of pigweed during the three periods of growth will show that the water requirement is not greatly affected by the period of growth.

If this holds for the other weeds, no great error will be produced in comparing the water requirement of these plants without regard to the period during which they were grown. The water requirement is, however, probably slightly less than it would have been if the plants had been grown in midsummer. The results obtained, based on the production of dry matter, are as follows:

Variety of weed	Water requirement
Purslane (*Portulaca oleracea*)	292±11
Pigweed (*Amaranthus retroflexus*)	320±7
Cocklebur (*Xanthium commune*)	432±13
Narrow-leaved sunflower from sand hills (*Helianthus petiolaris*)	570±11
Annual sunflower (*Helianthus annuus*)	705±8
Narrow-leaved sunflower from near Akron (*Helianthus petiolaris*)	774±20
Lamb's-quarters (*Chenopodium album*)	801±41
Fetid marigold (*Boebera papposa*)	881±26
Western ragweed (*Ambrosia artemisifolia*)	948±66

Purslane and pigweed, two introduced weeds, appear to be exceptionally efficient plants, their water requirement being only slightly higher than that of Kursk millet and in practical agreement with the sorghums. Some of the indigenous weeds were also found to be fairly efficient, cocklebur, a plant found in stream beds and about ponds, having a water requirement 13 per cent less than wheat, while the narrow-leaved sunflower from the sand hills had a water requirement 31 per cent less than alfalfa. Lamb's-quarters, an introduced plant, and fetid marigold (Pl. VII, fig. 2) and western ragweed, indigenous plants, have a slightly higher water requirement than alfalfa.

It is evident, therefore, that the common weeds differ greatly in water requirement. A growth of weeds in a crop or on summer fallow represents a tremendous loss of moisture, a thousand pounds per acre of the most efficient weeds representing a loss of at least 1.5 inches of stored rainfall, or from 4 to 5 inches of stored rainfall in the case of the weeds having a high water requirement. The latter figures represent about the maximum amount of moisture that can be stored in fallow land. It is therefore easy to understand how the whole of the stored moisture supply may be lost through the growth of a moderate crop of weeds, and these varieties having a high water requirement are especially to be dreaded.

TABLE XXVIII.—*Water requirement of weeds at Akron, Colo., in 1913*

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1913.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Sunflower (*Helianthus annuus*), June 5 to Sept. 15	271	516.6	40.7	373.5	8	9,180	723
	272	554.0	70.5	363.9	13	5,161	657
	273	502.2	51.8	343.2	10	6,625	683
	274	459.2	58.4	334.9	13	5,725	729
	275	512.5	63.0	371.6	12	5,900	724
	276	507.2	63.2	360.9	12	5,710	711
Mean						6,384±383	705±8
Sunflower, narrow-leaved (*Helianthus petiolaris*), July 25 to Sept. 17	175	196.0	34.1	135.5	17	3,971	691
	176	140.4	19.6	103.1	21	5,261	735
	177	148.7	23.3	109.5	16	4,695	736
	178	103.4	16.7	87.1	16	5,218	842
	179	156.0	21.8	132.2	14	6,061	848
	180	163.7	25.2	129.6	15	5,141	792
Mean						5,058±132	774±20
Sunflower, narrow-leaved (*Helianthus petiolaris*), from Sand hills, Aug. 13 to Sept. 18	79	17.1	.....	10.0	.....	.....	585
	80	32.3	.....	18.6	.....	.....	576
	81	34.6	.....	18.2	.....	.....	526
	82	14.4	.....	9.1	.....	.....	632
	83	25.4	.....	13.5	.....	.....	531
	84	30.5	.....	17.3	.....	.....	567
Mean							570±11
Marigold, fetid (*Boebera papposa*), July 25 to Sept. 17	169	116.0	.....	95.8	.....	.....	826
	170	102.8	.....	98.8	.....	.....	960
	171	106.5	.....	93.3	.....	.....	876
	172	99.9	.....	86.7	.....	.....	868
	173	167.3	.....	125.5	.....	.....	750
	174	55.0	.....	55.2	.....	.....	1,004
Mean							881±26
Lamb's-quarters (*Chenopodium album*), July 25 to Sept. 17	211	64.0	.....	39.9	.....	.....	624
	212	35.3	.....	26.9	.....	.....	762
	213	47.4	.....	46.2	.....	.....	975
	214	40.0	.....	33.4	.....	.....	835
	215	51.6	.....	47.2	.....	.....	914
	216	64.2	.....	44.6	.....	.....	694
Mean							801±41
Ragweed, western (*Ambrosia artemisiifolia*), Aug. 1 to Sept. 18	187	17.1	.....	12.5	.....	.....	731
	188	12.3	.....	12.9	.....	.....	1,049
	189	10.6	.....	13.8	.....	.....	1,302
	190	20.6	.....	18.7	.....	.....	908
	191	13.1	.....	13.3	.....	.....	1,015
	192	27.5	.....	18.7	.....	.....	680
Mean							948±66

TABLE XXVIII.—*Water requirement of weeds at Akron, Colo., in 1913*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirement based on—	
						Grain.	Dry matter.
1913.		*Grams.*	*Grams.*	*Kilos.*	*Per cent.*		
Pigweed (*Amaranthus retroflexus*), June 12 to July 19	91	56.9	.....	18.8	.....	.....	330
	92	133.7	.....	50.2	.....	.....	377
	93	145.7	.....	44.6	.....	.....	306
	94	115.0	.....	33.7	.....	.....	293
	95	112.8	.....	33.5	.....	.....	297
	96	161.5	.....	55.7	.....	.....	345
Mean	.....	.....	.....	.....	.....	.....	325±10
Pigweed, second crop, July 19 to Sept. 1	91	74.9	.....	22.5	.....	.....	300
	92	62.2	.....	20.1	.....	.....	323
	93	50.0	.....	16.4	.....	.....	328
	94	54.9	.....	17.9	.....	.....	326
	95	60.9	.....	20.5	.....	.....	337
	96	50.6	.....	17.2	.....	.....	340
Mean	.....	.....	.....	.....	.....	.....	326±4
Pigweed, third crop, Sept. 1 to Oct. 4	91	7.5	.....	2.2	.....	.....	293
	92	11.6	.....	3.6	.....	.....	310
	93	11.8	.....	3.4	.....	.....	288
	94	16.7	.....	4.2	.....	.....	251
	95	14.9	.....	3.9	.....	.....	262
	96	18.6	.....	4.9	.....	.....	263
Mean	.....	.....	.....	.....	.....	.....	278±7
Pigweed, combined crops, June 12 to Oct. 4	91	139.3	.....	43.5	.....	.....	312
	92	207.5	.....	73.9	.....	.....	356
	93	207.5	.....	64.4	.....	.....	310
	94	186.6	.....	55.8	.....	.....	299
	95	188.6	.....	57.9	.....	.....	307
	96	230.7	.....	77.8	.....	.....	337
Mean	.....	.....	.....	.....	.....	.....	320±7
Purslane (*Portulaca oleracea*), July 18 to Sept. 17	183	103.1	.....	33.2	.....	.....	322
Purslane, Aug. 21 to Sept. 17	241	36.2	.....	8.9	.....	.....	246
	242	28.0	.....	7.5	.....	.....	268
	243	33.5	.....	9.7	.....	.....	289
	244	38.4	.....	10.9	.....	.....	284
	245	48.5	.....	12.9	.....	.....	266
	246	18.8	.....	7.0	.....	.....	372
Mean	.....	.....	.....	.....	.....	.....	292±11

RELATIVE WATER-REQUIREMENT MEASUREMENTS

The relative water requirement of the different varieties of plants grown at Akron in 1912 is summarized in Table XXIX, Kubanka wheat being used as a basis of comparison. Grimm alfalfa is seen to have the highest water requirement of the 42 species and varieties tested during 1912, while Kursk millet proved the most efficient of all the plants tested. The varieties are also grouped on the basis of crop or genus, and their mean water requirement compared with the mean water require-

ment of the wheat varieties. The relative water requirement on this basis is given in the last column of Table XXIX.

The quantity of water required by the various crops for the production of a unit amount of seed or grain at Akron in 1912 is summarized in Table XXIV. Rye is seen to be the least efficient of the grain-producing crops tested, with Voronezh proso the most efficient.

TABLE XXIX.—*Summary of water-requirement measurements at Akron, Colo., in 1912*

WATER REQUIREMENT BASED ON DRY MATTER PRODUCED

Crop and variety.	Botanical name.	Water requirement of—			
		Variety.		Crop (or genus).	
		Actual.	Relative, compared with Kubanka wheat.	Actual.	Relative, compared with wheat (*Triticum* spp.).
1912.					
Alfalfa:					
Grimm, S. P. I. 25695.	Medicago sativa	659±6	1.67±0.03	658	1.60
Grimm, A. D. I. E. 23–20–52.	do	657±11	1.67± .04		
Clover, sweet	Melilotus alba	638±4	1.62± .03	638	1.56
Rice, Honduras	Oryza sativa	519±13	1.32± .04	519	1.27
Chick-pea	Cicer arietinum	510±14	1.29± .04	510	1.24
Rye, spring	Secale cereale	496±9	1.26± .03	496	1.21
Cotton, Triumph	Gossypium hirsutum.	488±14	1.24± .04	488	1.19
Native plants	Artemisia frigida	474±14	1.20± .04	471	1.15
	Grindelia squarrosa	468±18	1.19± .05		
Oats:					
Sixty-Day	Avena sativa	491±13	1.25± .04	441	1.08
Burt	do	449±3	1.14± .02		
Swedish Select	do	423±5	1.07± .02		
Canadian	do	399±6	1.01± .02		
Barley:					
Hannchen	Hordeum distichon	443±3	1.12± .02	425	1.04
White Hull-less	Hordeum vulgare	439±1	1.11± .02		
Beldi	do	416±4	1.06± .02		
Beardless	do	403±8	1.02± .03		
Wheat:					
Spring Ghirka	Triticum aestivum	457±3	1.16± .02	410	1.00
Marvel Bluestem	do	451±4	1.14± .02		
Emmer	Triticum dicoccum	428±3	1.09± .02		
Kubanka	Triticum durum	394±7	1.00		
Kharkov	Triticum aestivum	365±6	.93± .02		
Turkey	do	364±6	.92± .02		
Sugar beet:					
Kleinwanzleben	Beta vulgaris	321±8	.81± .03	321	.78
Corn:					
China White	Zea mays	315±7	.80± .02	286	.70
Iowa Silvermine	do	302±7	.77± .02		
Laguna	do	295±6	.75± .02		
China White×Laguna.	do	289±4	.73± .02		
Hopi	do	285±7	.72± .02		
Northwestern Dent.	do	280±10	.71± .03		
China White×Esperanza.	do	250±2	.63± .01		
Esperanza	do	239±3	.61± .01		

TABLE XXIX.—*Summary of water-requirement measurements at Akron, Colo., in 1912*—Continued

WATER REQUIREMENT BASED ON DRY MATTER PRODUCED—Continued

Crop and variety.	Botanical name.	Water requirement of—			
		Variety.		Crop (or genus).	
		Actual.	Relative, compared with Kubanka wheat.	Actual.	Relative, compared with wheat (*Triticum* spp.).
1912.					
Sorghum:					
Sudan grass	Andropogon sorghum aethiopicus.	359±2	0.91±0.02	262	0.64
Milo, Dwarf	Andropogon sorghum	273±4	.69± .02		
Kafir, Blackhull	do	259±5	.66± .02		
Durra, White	do	255±3	.65± .02		
Milo	do	249±3	.63± .02		
Minnesota Amber	do	239±2	.61± .01		
Red Amber	do	237±4	.60± .01		
Kaoliang, Brown	do	223±1	.57± .01		
Millet:					
German	Chaetochloa italica	248±7	.63± .02	218	.53
Kursk	do	187±2	.47± .01	207	.51
Proso:					
Tambov	Panicum miliaceum	208±1	.53± .01	207	.51
Voronezh	do	206±1	.52± .01		

WATER REQUIREMENT BASED ON GRAIN OR SEED PRODUCED

Rye, spring	Secale cereale	1,802±62	1.61±0.08	1,802	1.50
Chick-pea	Cicer arietinum	1,348±114	1.21± .11	1,348	1.13
Oats:					
Canadian	Avena sativa	1,416±119	1.27± .12	1,229	1.03
Burt	do	1,224±55	1.10± .07		
Sixty-Day	do	1,172±133	1.05± .12		
Swedish Select	do	1,103±18	.99± .04		
Wheat:					
Marvel Bluestem	Triticum aestivum	1,573±49	1.42± .06	1,197	1.00
Spring Ghirka	do	1,468±34	1.32± .06		
Kubanka	Triticum durum	1,111±37	1.00		
Kharkov	Triticum aestivum	1,064±60	.96± .06		
Turkey	do	995±22	.90± .04		
Emmer	Triticum dicoccum	984±18	.89± .03		
Barley:					
White Hull-less	Hordeum vulgare	1,239±11	1.11± .04	1,051	.88
Beardless	do	1,017±83	.92± .08		
Hannchen	Hordeum distichon	1,005±36	.90± .04		
Beldi	Hordeum vulgare	941±10	.84± .03		
Sorghum:					
Kaoliang, Brown	Andropogon sorghum.	927±38	.83± .04	767	.64
Minnesota Amber	do	607±15	.55± .02		
Millet:					
Kursk	Chaetochloa italica	483±11	.43± .02	483	.41
Proso:					
Tambov	Panicum miliaceum	482±9	.43± .02	454	.38
Voronezh	do	425±4	.38± .01		

The relative water requirement of the different varieties included in the 1913 experiments will be found summarized on the basis of dry matter in Table XXX and on the basis of grain production in Table XXXI. Fifty-five species and varieties were included in these measurements. Reference to these tables will show that a number of plants had a higher water requirement than alfalfa, heretofore the most inefficient in the use of water of any plant included in these experiments. On the other hand, millet maintains its supremacy as the most efficient plant so far included in the water-requirement measurements.

TABLE XXX.—*Summary of water-requirement measurements at Akron, Colo., in 1913, based on dry matter produced*

Crop and variety.	Botanical name.	Water requirement.	Relative, as compared with Kubanka wheat.
1913.			
Wheat-grass, western	Agropyron Smithii	1,076±29	2.17±0.06
Brome-grass	Bromus inermis	1,016±26	2.10± .06
Ragweed, western	Ambrosia artemisifolia	948±66	1.91± .13
Vetch, purple	Vicia atropurpurea	935±9	1.89± .03
Flax	Linum usatissimum	905±25	1.82± .05
Marigold, fetid	Boebera papposa	881±26	1.78± .06
Pumpkin	Cucurbita pepo	834±17	1.68± .04
Alfalfa, Grimm	Medicago sativa	834±8	1.68± .02
Soy bean, wild	Glycine soja	815±25	1.64± .05
Clover, crimson	Trifolium incarnatum	805±8	1.62± .02
Lamb's-quarters	Chenopodium album	801±41	1.62± .08
Clover, red	Trifolium repens	789±9	1.59± .02
Bean, horse, S. P. I. 15429	Vicia faba	780±19	1.57± .04
Pea, Canada field	Pisum sativum	775±5	1.56± .02
Sunflower, narrow leaved	Helianthus petiolaris	774±20	1.56± .04
Bean, Mexican	Phaseolus vulgaris	773±8	1.56± .02
Bean, horse, S. P. I. 25645	Vicia faba	772±11	1.56± .03
Squash	Cucurbita maxima	748±8	1.51± .02
Rice, Honduras	Oryza sativa	744±17	1.50± .04
Rape	Brassica napus	743±7	1.50± .02
Potato, McCormick	Solanum tuberosum	717±11	1.45± .03
Cucumber	Cucumbis sativa	713±11	1.44± .03
Sunflower, annual	Helianthus annuus	705±8	1.42± .02
Wheat-grass	Agropyron cristatum	705±27	1.41± .06
Vetch, hairy	Vicia villosa	690±8	1.39± .02
Bean, navy	Phaseolus vulgaris	682±4	1.38± .02
Bean, soy, cultivated	Glycine hispida	672±9	1.35± .02
Potato, Irish Cobbler	Solanum tuberosum	659±15	1.33± .03
Cotton, Triumph	Gossypium hirsutum	657±11	1.33± .03
Alfalfa, Peruvian	Medicago sativa	651±12	1.31± .03
Turnip	Brassica rapa	639±31	1.29± .06
Cantaloupe	Cucumis melo	621±27	1.25± .06
Oat:			
Swedish Select	Avena sativa	617±9	1.24± .02
Burt	do	617±5	1.24± .02
Watermelon	Citrullus vulgaris	600±15	1.21± .03
Cowpea	Vigna sinensis	571±3	1.15± .01
Sunflower, narrow-leaved, from sand hills.	Helianthus petiolaris	570±11	1.15± .03
Cabbage	Brassica oleracea capitata	539±7	1.09± .02
Wheat, Kubanka	Triticum durum	496±5	1.00
Cocklebur	Xanthium commune	432±13	.87± .03

TABLE XXX.—*Summary of water requirement measurements at Akron, Colo., in 1913, based on dry matter produced*—Continued

Crop and variety.	Botanical name.	Water requirement.	Relative as compared with Kubanka wheat.
1913.			
Corn:			
China White	Zea mays	415±4	0.84±0.01
Bloody Butcher	do	405±7	.82± .02
Northwestern Dent	do	399±12	.80± .03
Teosinte, Durango	Euchlene mexicana	390±11	.79± .02
Grass, buffalo and grama	Bulbilis dactyloides and Boutelona gracilis.	389±12	.78± .03
China White×Teosinte		376±4	.76± .01
Corn:			
Hopi	Zea mays	350±8	.71± .02
China White×Hopi	do	345±3	.70± .01
Indian Flint	do	342±5	.69± .01
Pigweed	Amaranthus retroflexus	320±7	.65± .02
Grass, buffalo	Bulbilis dactyloides	308±17	.61± .04
Sorghum:			
Minnesota Amber	Andropogon sorghum	298±2	.60± .01
Red Amber	do	296±1	.59± .01
Purslane	Portulaca oleracea	292±11	.59± .02
Millet, Kursk	Chaetochloa italica	286±4	.58± .01

TABLE XXXI.—*Summary of water-requirement measurements in 1913 based on grain, tubers, roots, or fruit produced*

Crop and variety.	Botanical name.	Water requirement.	Relative water requirement compared with Kubanka wheat.
1913.			
Sunflower:			
Annual	Helianthus annuus	6,384±383	4.83±0.29
Narrow-leaved	Helianthus petiolaris	5,058±132	3.83± .11
Flax	Linum usitatissimum	2,835±52	2.14± .05
Pea, Canada field	Pisum sativum	2,322±121	1.76± .11
Bean, soy	Glycine soja	2,053±51	1.55± .04
Potato, Irish Cobbler	Solanum tuberosum	2,027±197	1.53± .16
Bean, Mexican	Phaseolus vulgaris	1,888±62	1.43± .05
Oats, Swedish Select	Avena sativa	1,876±55	1.41± .04
Cantaloupe	Cucumis melo	1,824±237	1.38± .18
Bean, Navy	Phaseolus vulgaris	1,646±36	1.25± .03
Oats, Burt	Avena sativa	1,641±33	1.24± .03
Cucumber	Cucumis sativa	1,611±67	1.22± .05
Cowpea	Vigna sinensis	1,576±32	1.19± .03
Turnip	Brassica campestris	1,530±132	1.16± .10
Wheat, Kubanka	Triticum durum	1,322±16	1.00
Corn, Northern Dent	Zea mays	1,241±77	.94± .06
Watermelon	Citrullus vulgaris	1,146±49	.87± .04
Sorghum, Red Amber	Andropogon sorgum	1,100±31	.83± .03
Corn, Indian Flint	Zea mays	854±31	.65± .01
Sorghum, Minnesota Amber	Andropogon sorghum	765±12	.58± .01

COMPARISON OF THE WATER REQUIREMENT OF CROPS AT AKRON, COLO., IN 1911, 1912, AND 1913

Climatic conditions at Akron during the summer of 1912 were less severe than during the preceding summer. The rainfall in 1912 was much greater than in 1911, the temperature was lower, and the evaporation was less. These conditions were apparently due in part to a marked reduction in the intensity of the solar radiation at the earth's surface following the eruption of Mount Katmai, Alaska, early in June, 1912, the dust from which produced a haze in the upper atmosphere. Abbot and Fowle (1913) observed a maximum reduction in the solar radiation of about 20 per cent at Bassour, Algeria, and at Mount Wilson, Cal., Kimball (1913a, b) reports an average reduction of 17 per cent in the intensity of the solar radiation at Mount Weather, Va., during the last half of 1912, while Briggs and Belz (1913) have shown that there was a general reduction in the evaporation from a free-water surface during the summer months following the eruption. It is consequently of interest to determine whether the diminution in the intensity of the solar radiation was accompanied by a reduction in the water requirement in 1912. Such a comparison is possible in connection with the Akron experiments, since a large number of the varieties employed in the experiments of 1911 were also included in the 1912 measurements. All varieties showed in 1912 a marked reduction in the water requirement as compared with 1911. The measurements for each year are given in Table XXXII, together with the ratio of the 1912 to the 1911 measurements. The 1912 measurements show an average reduction in the water requirement of 21 ± 2 per cent for the 25 varieties tested during both years. The individual ratios fluctuate somewhat, doubtless owing in part to errors of experiments,[1] but in part also to the different response of individual varieties to changed climatic conditions.

[1] It should be mentioned here that the plants were fertilized in 1912 and not in 1911. This is a matter of importance in this connection, because it is well established that any deficiency in plant food increases the water requirement. The effect of the addition of fertilizer on the water requirement was measured both years. The use of fertilizer resulted in a slight reduction (6 ± 2.3 per cent) in the water requirement of Kubanka wheat at Akron in 1911, comparing pots 7 to 12, fertilized, with pots 1 to 6, unfertilized. These pots stood side by side in the inclosure. (*See* Briggs and Shantz, 1913a, p. 19.) In 1912 the fertilized Kubanka wheat plants showed a slight increase in water requirement—namely, 5 ± 2.3 per cent, comparing pots 1 to 6 against pots 7 to 12. The differences in each instance are without significance when the errors are considered and are furthermore of opposite sign, so that the addition of fertilizer may be considered to have had no effect on the water requirement, so far as Kubanka wheat was concerned. Rich surface soil from the same source was employed in the experiments of both years.

TABLE XXXII.—*Comparison of water-requirement measurements at Akron, Colo., in 1911 and 1912*

Crop.	Water requirement.		Ratio, 1912 to 1911.
	1911.	1912.	
Wheat:			
Kubanka	468±8	394±7	0.84±0.02
Bluestem	531±5	451±4	.85± .01
Spring Ghirka	506±3	457±3	.90± .01
Emmer	534±14	428±3	.80± .02
Oats:			
Sixty-Day	605±5	491±13	.81± .02
Burt	639±7	449±3	.70± .01
Canadian	598±14	399±6	.67± .02
Swedish Select	615±7	423±5	.69± .01
Barley:			
Hannchen	527±8	443±3	.84± .01
Beldi	543±2	416±4	.76± .01
White Hull-less	542±3	439±1	.81± .01
Beardless	544±9	403±8	.74± .02
Rye, spring	724±7	496±9	.69± .01
Corn:			
Northwestern Dent	368±10	280±10	.75± .03
Iowa Silvermine	420±3	302±7	.72± .02
Esperanza	319±5	239±3	.79± .01
Sorghum:			
Red Amber	298±4	237±4	.80± .02
Milo, Dwarf	333±3	273±4	.82± .02
Kafir, Blackhull	278±5	259±5	.93± .02
Durra, White	321±2	255±3	.79± .01
Kaoliang, Brown	301±3	223±1	.74± .01
Millet, German	263±15	248±7	.94± .05
Legumes:			
Alfalfa	1,068±16	659±6	.62± .03
Clover, sweet	709±9	638±4	.90± .02
Beet, sugar	377±8	321±8	.85± .03
Mean water-requirement ratio for 1912 to 1911			.79± .02
Mean evaporation ratio for June, July, and August, 1912 to 1911			.75± .03

Evaporation measurements from a free-water surface at Akron are also available for 1911 and 1912 (Briggs and Belz, 1910, p. 17). The ratio of the evaporation in 1912 to that in 1911 by months is as follows: June, 0.69; July, 0.78; August, 0.79. The average ratio during these three months is 0.75±0.03. This corresponds to a reduction of 25±3 per cent in evaporation as compared with a reduction of 21±2 per cent in the water requirement. The change in the water requirement of these 25 varieties taken together is seen to be in approximate agreement with the change in evaporation from a free-water surface. A consideration of the individual ratios indicates, however, that different varieties may respond quite differently to the same change in climatic conditions.

To investigate further the variation in water requirement due to differences in climatic conditions, a number of varieties were also

included in the measurements of both 1912 and 1913. The ratios of the water requirement of these crops in 1912 to that in 1913 are given in Table XXXIII. Similar ratios are also given for crops grown in 1911 and 1913. It will be seen that the mean 1913–1911 ratio approximates unity; in other words, the mean water requirement of the crops under investigation was practically the same for both years. The water requirement in 1912 is seen to be far below the 1913 value, the mean ratio being 0.75±0.01. The mean ratio of the monthly evaporation for June, July, and August, 1912, compared with 1913, is 0.80± 0.02, which is in approximate agreement with the ratio of the water requirement of crops grown during the two years.

The crops at Akron as influenced by climatic conditions in 1911, 1912, and 1913 may then be summarized as follows:

TABLE XXXIII.—*Comparison of the water requirement of the same crops at Akron, Colo., 1911, 1912, and 1913*

Crop.	Water requirement.				
				Ratio.	
	1911.	1912.	1913.	1913 to 1911.	1913 to 1912.
Wheat, Kubanka	468±8	394±7	496±5	1.06	0.80
Oats:					
Swedish Select	615±7	423±5	617±9	1.00	.69
Burt	639±7	449±3	617±5	.97	.73
Corn:					
Northwestern Dent	368±10	280±10	399±12	1.08	.70
Hopi	.....	285±7	358±8	.....	.80
China White	.....	315±7	416±4	.....	.76
Sorghum:					
Minnesota Amber	.....	239±2	298±2	.....	.80
Red Amber	298±4	237±4	296±1	.99	.80
Millet, Kursk	.....	187±2	286±4	.....	.66
Legumes:					
Alfalfa, Grimm	1,068±16	657±11	834±8	.77	.79
Pea, Canada field	800±17	.....	775±5	.97	.....
Potato, Irish Cobbler	448±11	.....	659±15	1.47	.....
Cotton	.....	488±14	657±11	.....	.75
Rice	.....	496±9	744±17	.....	.67
Mean water requirement ratio	.....	.....	.....	1.04±.04	.75±01
Evaporation in inches for three summer months	28.46	21.42	26.75	.94±.04	.80±02

The conditions during 1911 and 1913 were such as to give rise to practically the same water requirement. The water requirement of crops grown in 1912 was on the average only 79±2 per cent of crops grown in 1911 and 75±2 per cent of crops grown in 1913. Therefore, in order to determine the relative water requirement of the different crops, it appears justifiable to increase the 1912 water requirement ratios by the reciprocal of 0.77—namely, 1.3. This procedure has been followed in the summary

table (Table XXXIV), which places the water requirement of all crops upon the basis of years similar to 1911 and 1913. When the water-requirement measurement of any particular crop extended over more than one year, the mean value of the several water-requirement determinations is given in Table XXXIV.

SUMMARY

This paper deals with the measurement of the water requirement of plants at Akron, Colo., in the central portion of the Great Plains. The term "water requirement" is here used to express the ratio of the water absorbed by a plant during its period of growth to the dry matter produced. The plants were grown to maturity in large galvanized-iron pots having a capacity of about 115 kg. of soil. Each pot was provided with a tight-fitting cover having openings for the stems of the plants, the annular space between the stem of the plant and the cover being sealed with wax. The loss of water was thus practically confined to that taking place through transpiration, and the entrance of rainfall was almost wholly prevented. The pots were weighed two or three times weekly to determine the amount of water required to maintain normal weight. Water was delivered from 2-liter calibrated flasks through stoppered openings in the middle of the cover to a 5-inch flowerpot sunk in the soil immediately beneath the cover.

To protect the plants from birds and severe wind and hail storms, it was found necessary to conduct the experiments in a screened inclosure. Pyrheliometric measurements showed that the inclosure reduced the radiation about 20 per cent. Water-requirement measurements conducted simultaneously with the same plants inside and outside the inclosure showed that the inclosure also reduced the water requirement approximately 20 per cent.

Rich surface soil was used in the pots, and the pots were also fertilized to insure an adequate supply of plant food. Six pots of plants of each variety were used, and the water requirement of each pot was determined independently, in order to provide a basis for the determination of the probable errors of the experiment.

The detailed results given in the paper comprise measurements of 44 species and varieties in 1912 and 55 in 1913. The writers' 1911 measurements have also been included in the summary table. The years 1911 and 1913 were similar in character, and the same plants grown during both years gave practically the same water requirement. The year 1912 was cooler and the evaporation and light intensity were much lower. These conditions had a marked influence on the water requirement, the mean water requirement in 1912 being only 77 per cent of that in 1911 and 1913. In order to place all of the determinations upon a comparative basis, the 1912 measurements have accordingly been increased 30 per cent in the summary table (Table XXXIV).

TABLE XXXIV.—*Summary of water-requirement determinations at Akron, Colo., in 1911, 1912, and 1913, based on the production of dry matter*

Plant.	Botanical name.	Number of observations.	Water requirement.	
			Of species or variety.	Mean of genus.
GRAIN CROPS.		*Years.*		
Proso:				
Voronezh, C. I. 16	Panicum miliaceum	1	268±1	293
Tambov, S. D. 366	do	1	270±1	
Black Voronezh, S. D. 334	do	1	341±10	
Millet:				
Kursk, S. P. I. 30029	Chaetochloa italica	1	261±15	310
Kursk, S. P. I. 34771	do	2	265±3	
Kursk, S. P. I. 22420	do	1	287±2	
German, S. P. I. 26845	do	2	293±9	
Turkestan, S. P. I. 20694	do	1	444±9	
Sorghum:				
Kafir, Dwarf Blackhull, C. I. 340.	Andropogon sorghum	1	285±3	322
Kaoliang, Brown, S. P. I. 24993.	do	2	296±2	
Kafir, White, C. I. 370	do	1	297±4	
Red Amber, S. P. I. 17563	do	3	301±2	
Kafir, Early Blackhull, C. I. 472.	do	1	302±13	
Minnesota Amber, A. D. I. 341–13.	do	2	305±2	
Kafir, Blackhull, S. P. I. 24975.	do	2	308±4	
Milo, White, C. I. 365	do	1	317±3	
Kafir×Durra, C. I. 198–15–3.	do	1	321±5	
Feterita, C. I. 182	do	1	323±4	
Milo, S. P. I. 24960	do	1	324±4	
Durra, White, S. P. I. 24997.	do	1	327±2	
Milo, Dwarf, S. P. I. 24970.	do	2	344±3	
Sudan grass, S. P. I. 25071.	do	1	467±9	
Corn:				
Esperanza	Zea mays	2	315±3	368
China White×Esperanza	do	1	325±2	
Indian Flint	do	1	342±5	
China White×Hopi	do	1	345±3	
Hopi	do	2	361±6	
China White×Laguna	do	1	376±5	
Northwestern Dent	do	3	377±7	
Laguna	do	1	384±8	
Bloody Butcher	do	1	405±7	
Iowa Silvermine	do	2	407±5	
China White	do	2	413±5	
Teosinte:				
China White×Teosinte		1	376±4	383
Teosinte	Euchlaena mexicana	1	390±11	
Wheat:				
Turkey, C. I. 1571	Triticum aestivum	1	473±8	513
Kharkov, C. I. 1583	do	1	475±8	
Kubanka, C. I. 1440	Triticum durum	3	492±4	
Galgalos, C. I. 2398	Triticum aestivum	1	496±4	
Emmer, C. I. 2951	Triticum dicoccum	2	545±7	
Spring Ghirka, C. I. 1517	Triticum aestivum	2	550±3	
Marvel Bluestem, C. I. 3082	do	2	559±4	

TABLE XXXIV.—*Summary of water-requirement determinations at Akron, Colo., in 1911, 1912, and 1913, based on the production of dry matter*—Continued

Plant.	Botanical name.	Number of observations.	Water requirement. Of species or variety.	Water requirement. Mean of genus.
GRAIN CROPS—continued.				
Barley:		*Years.*		
Hannchen, C. I. 531	Hordeum distichon	2	502±4	534
Beardless, C. I. 716	Hordeum vulgare	2	534±7	
Beldi, C. I. 190	do	2	542±3	
White Hull-less, C. I. 595	do	2	556±2	
Buckwheat	Fagopyrum fagopyrum	1	578±13	578
Oats:				
Canadian, C. I. 444	Avena sativa	2	559±8	597
Swedish Select, C. I. 134	do	3	594±4	
Burt, C. I. 293	do	3	613±3	
Sixty-Day, C. I. 165	do	2	622±9	
Rye, spring, C. I. 73	Secale cereale	2	685±7	685
Rice, Honduras, C. I. 1643	Oryza sativa	2	710±15	710
Flax, North Dakota, No. 155	Linum usitatissimum	1	905±25	905
OTHER CROPS.				
Beet, sugar:				
Morrison-grown Kleinwanzleben.	Beta vulgaris	2	397±6	397
Potato:				
Irish Cobbler	Solanum tuberosum	2	554±9	636
McCormick	do	1	717±11	
Crucifers:				
Cabbage, Early Jersey, Wakefield.	Brassica oleracea capitata	1	539±7	640
Turnip, Purple-top	Brassica rapa	1	639±31	
Rape	Brassica napus	1	743±7	
Cotton, Triumph	Gossypium hirsutum	2	646±11	646
Cucurbits:				
Watermelon, Rocky Ford	Citrullus vulgaris	1	600±15	600
Cantaloupe, Rocky Ford	Cucumis melo	1	621±27	667
Cucumber, Boston Pickling	Cucumis sativa	1	713±11	
Squash, Hubbard	Cucurbita maxima	1	748±8	791
Pumpkin, common field	Cucurbita pepo	1	834±17	
Legumes:				
Cowpea, S. P. I. 29282	Vigna sinensis	1	571±3	571
Chick-pea, S. P. I. 24322	Cicer arietinum	1	663±18	663
Bean, navy	Phaseolus vulgaris	1	682±4	728
Bean, Mexican	do	1	773±8	
Bean, soy, S. P. I. 21755	Glycine hispida	1	672±9	744
Bean, soy (wild), S. P. I. 25138.	Glycine soja	1	815±25	
Clover, sweet, S. P. I. 21216	Melilotus alba	2	770±5	770
Pea, Canada field, S. P. I. 30134.	Pisum sativum	1	775±5	788
Pea, Canada field, S. P. I. 22637.	do	1	800±17	
Vetch, hairy, S. P. I. 34298	Vicia villosa	1	690±8	794
Bean, horse, S. P. I. 25645	Vicia faba	1	772±11	
Bean, horse, S. P. I. 15429	do	1	780±10	
Vetch, purple, S. P. I. 18131.	Vicia atropurpurea	1	935±9	
Clover, red, S. P. I. 34869	Trifolium repens	1	789±9	797
Clover, crimson, S. P. I. 33742.	Trifolium incarnatum	1	805±8	

TABLE XXXIV.—*Summary of water-requirement determinations at Akron, Colo., in 1911, 1912, and 1913, based on the production of dry matter*—Continued

Plant.	Botanical name.	Number of observations.	Water requirement.	
			Of species or variety.	Mean of genus.
OTHER CROPS—continued.				
Legumes—Continued.		*Years.*		
Alfalfa, Peruvian, S. P. I. 30203.	Medicago sativa	1	651±12	831
Alfalfa, Grimm, A. D. I. E–23–20–52.	.....do	2	844±8	
Alfalfa, yellow-flowered	Medicago falcata	1	865±18	
Alfalfa, Grimm, S. P. I. 25695.	Medicago sativa	2	963±9	
Grasses:				
Wheat-grass	Agropyron cristatum	1	705±27	861
Brome-grass	Bromus inermis	1	1,016±26	
NATIVE PLANTS.				
Tumbleweed	Amaranthus graecizans	1	277±4	287
Pigweed	Amaranthus retroflexus	2	297±4	
Purslane	Portulaca oleracea	1	292±11	292
Grass, buffalo	Bulbilis dactyloides	1	308±17	308
Thistle, Russian	Salsola pestifer	1	336±5	336
Grass, buffalo and grama	Bulbilis dactyloides / Bouteloua gracilis	1	389±12	389
Cocklebur	Xanthium commune	1	432±13	432
Gumweed	Grindelia squarrosa	1	608±23	608
Sage, mountain	Artemisia frigida	1	616±18	616
Sunflower (Akron)	Helianthus petiolaris	1	774±20	683
Sunflower (sand hills)	.....do	1	570±11	
Sunflower	Helianthus annuus	1	705±8	
Lamb's-quarters	Chenopodium album	1	801±41	801
Marigold, fetid	Boebera papposa	1	881±26	881
Ragweed, western	Ambrosia artemisifolia	1	948±06	948
Wheat-grass, western	Agropyron Smithii	1	1,076±29	1,076

The results given in the summary table (Table XXXIV) therefore represent the water requirement of the plants for years similar to 1911 and 1913, when grown in a screened inclosure, which reduces the solar radiation to 80 per cent of its normal value. According to measurements made with wheat, alfalfa, and cocklebur, the removal of the inclosure would increase the water requirement as given in the table by 25 per cent. The plants grown outside the inclosure were isolated and freely exposed, while plants under field conditions mutually protect and shade one another to some extent. Comparison with wheat plants grown in pots sunk in trenches indicates that the inclosure measurements, at least in the case of wheat, are less than 10 per cent below the water requirement of plants exposed under field conditions.

The measurements in Table XXXIV represent the relative water requirement of the different plants tested, subject to the limitations imposed by the difference in the growth period of the plants. The

measurements in 1912 would also indicate that the character of the season influences the water requirement of some plants more than others. For this reason, where the results of two or more years have been combined in the table, the probable error has been confined to the errors of experiment and does not include the fluctuations in water requirement due to the season.

In order to facilitate comparison, the plants have been arranged under three main heads: Grain crops, other crops, and native plants. Under "Grain crops" are also included certain sorghums and millets which are usually grown for forage. Under the heading "Other crops" are included principally the legumes, cucurbits, crucifers, sugar beets, cotton, and potatoes, as well as some of the introduced grasses. Under the heading "Native plants" are listed indigenous species, as well as certain introduced species which have become thoroughly established.

The grain crops fall rather naturally into two sections: Those of low water requirement—proso, millet, sorghum, and corn—and those of high water requirement—wheat, barley, oats, rye, and flax. The plants with a comparatively low water requirement are late-maturing crops, which make their best growth during the hottest and driest portion of the summer. The plants having a comparatively high water requirement mature during midsummer and make their best growth during the earlier, cooler period of the year. The range in water requirement of the first group is from 261 for Kursk millet to 468 for Sudan grass, while the range in the second group is from 473 for Turkey wheat to 905 for flax.

Representing the water requirement of proso as 1, the water requirement of the grain crops is as follows: Millet, 1.06; sorghum, 1.10; corn, 1.26; teosinte, 1.34; wheat, 1.76; barley, 1.83; buckwheat, 1.98; oats, 2.04; rye, 2.34; rice, 2.42; and flax, 3.38. In other words, flax requires more than three times as much water and rice more than twice as much water as proso and millet in producing a pound of dry matter.

In the second group sugar beet ranks first, having a water requirement almost as low as corn. Potato ranks next, followed by crucifers, cucurbits, legumes, and grasses, in order. A wide range is shown in each of these families. The groups as a whole show a range somewhat less than the grain groups.

Representing the water requirement of sugar beet as 1, the values for the "Other crops," exclusive of the legumes, are as follows: Cabbage, 1.36; Irish Cobbler potato, 1.39; watermelon, 1.51; cantaloupe, 1.57; turnip, 1.60; cotton, 1.63; cucumber, 1.80; wheat-grass, 1.85; rape, 1.87; squash, 1.89; pumpkin, 2.10; and brome-grass, 2.56.

The cowpea was the most efficient of the legumes. Representing its water requirement by 1, that of the other legumes is as follows: Peruvian alfalfa, 1.14; chick-pea, 1.16; soy bean, 1.18; navy bean, 1.20; hairy vetch, 1.21; sweet clover, 1.35; Mexican bean, 1.35; horse bean, 1.36; red clover, 1.38; Canada field pea, 1.38; crimson clover, 1.41; wild soy

bean, 1.42; select Grimm alfalfa, 1.48; yellow-flowered alfalfa, 1.51; purple vetch, 1.64; and unselected Grimm alfalfa, 1.69.

The native plants show a range in water requirement greater even than the cultivated crops. Amaranths, buffalo and grama grasses, purslane, and Russian thistle have a low water requirement and compare favorably with millet and sorghum, while sunflower, fetid marigold, western ragweed, and western wheat-grass have a high water requirement about equal to that of alfalfa.

Representing the water requirement of tumbleweed (*Amaranthus graecizans*) as unity, the water requirement of these native plants is as follows: Purslane, 1.05; pigweed, 1.07; buffalo grass, 1.11; Russian thistle, 1.21; buffalo and grama grasses, 1.40; cocklebur, 1.56; gumweed, 2.20; mountain sage, 2.22; sunflower, 2.56; narrow-leaved sunflower, 2.80; lamb's-quarters, 2.89; fetid marigold, 3.18; western ragweed, 3.42; western wheat-grass, 3.89.

Varieties of the same crop often differ widely in water requirement. In the case of barley, the variety having the highest water requirement was 8 per cent above the lowest; oats, 11 per cent; wheat, 18 per cent; proso, 27 per cent; corn, 31 per cent; vetch, 35 per cent; alfalfa, 48 per cent; sorghum, 60 per cent; and millet, 70 per cent. This wide range in water requirement among the varieties of many crops encourages the belief that strains may yet be secured which are still more efficient in the use of water than those now grown in dry-land regions.

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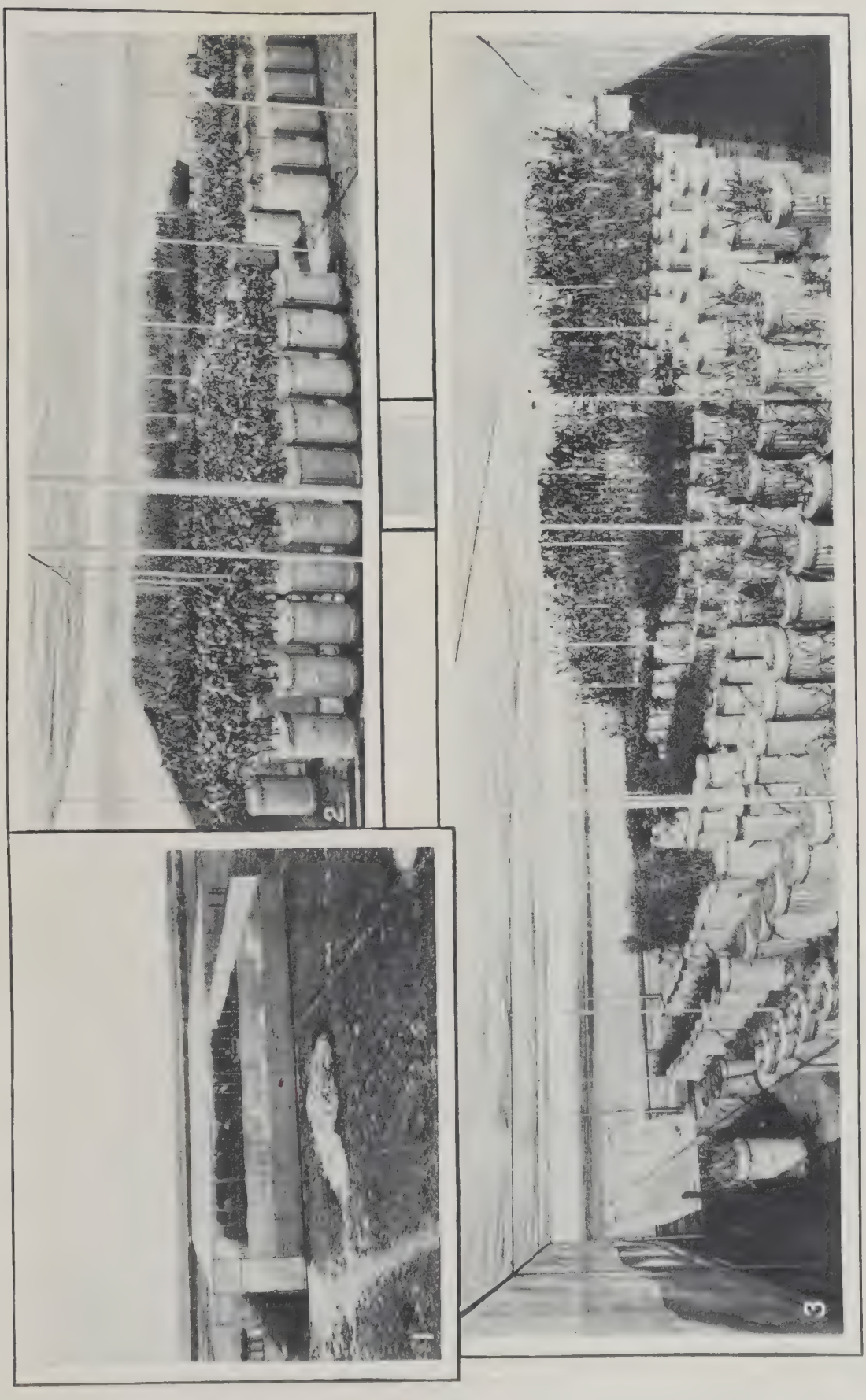
PLATE I

Fig. 1.—General view of the plant inclosure used at Akron, Colo., showing the pipe framework covered with a hail screen, with the board base surmounted by a single width of cheesecloth to protect the plants against high winds. Photographed on July 15, 1912.

Fig. 2.—General view inside the inclosure, showing the arrangement of pots and general conditions of growth. Corn and sorghums are shown in the foreground, small grain in the background. Photographed on July 3, 1912.

Fig. 3.—General view of the inclosure photographed shortly after the grain in some of the pots had been harvested. Photographed on September 4, 1912.



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2
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PLATE II

Fig. 1.—Pot planted with sugar beets, showing the wax seal around the plants and also the sealed holes where stand was not perfect.

Fig. 2.—Weighing pots, showing spring balance, weighing support, and general procedure. Two men operate the weighing support, one of whom lifts the pot by means of a windlass, while a third reads the balance and records the weight. By this method weighings can be made at the rate of two per minute.

Fig. 3.—*Grindelia squarrosa* (gumweed) at left (pot 164), and *Artemisia frigida* (mountain sage) at right (pot 167), illustrating the growth of native plants used in the water-requirement measurements. Photographed on July 29, 1912. Water requirement of Grindelia, 468±18; of Artemisia, 474±14.

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PLATE III

Fig. 1.—Kubanka wheat (pots 1 to 6), grown May 9 to September 3, 1912. Photographed on July 19, 1912. Water requirement, 394±7.

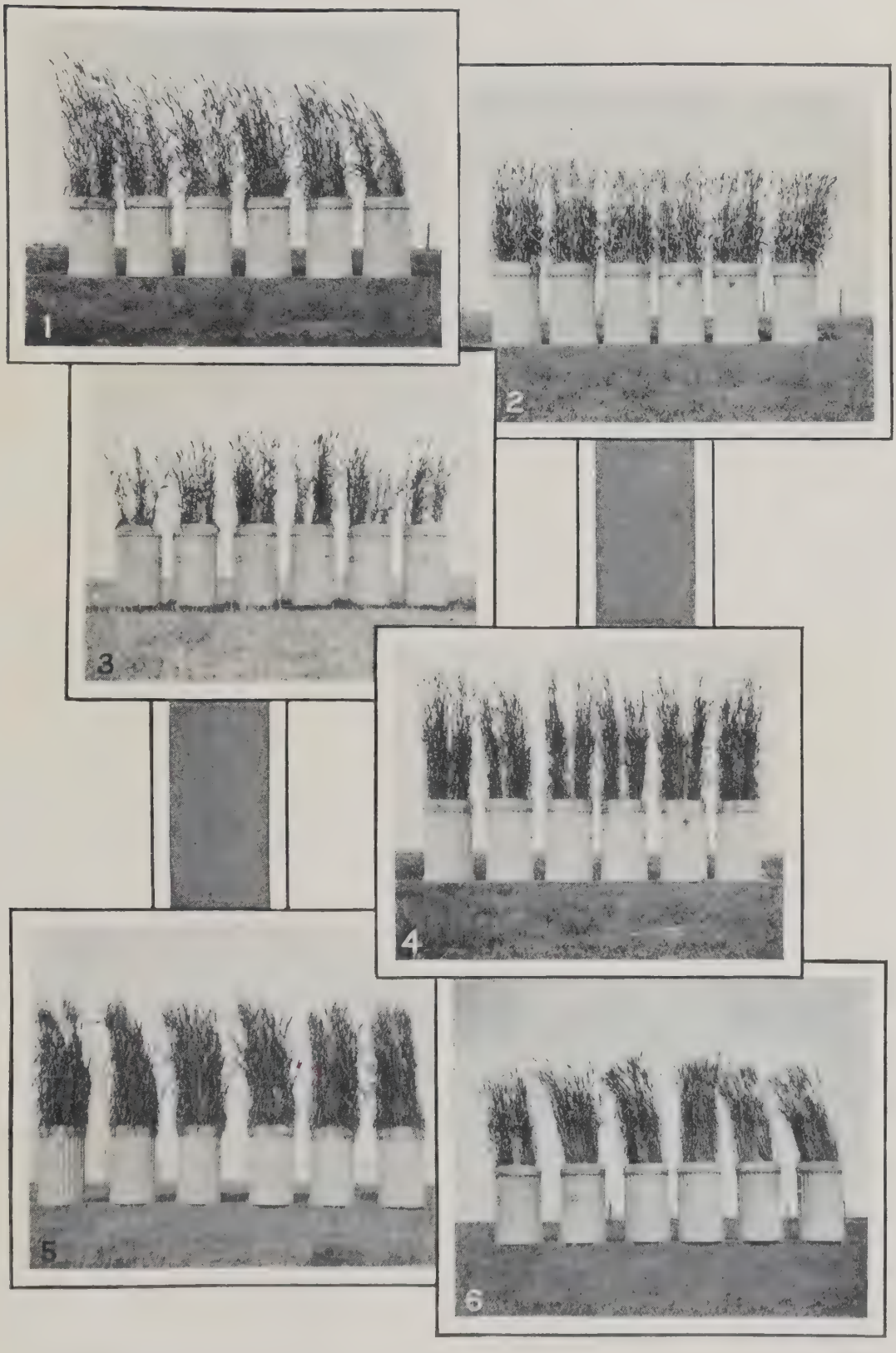
Fig. 2.—White Hull-less barley (pots 103 to 108), grown May 16 to August 12, 1912. Photographed on July 19, 1912. Water requirement, 439±1.

Fig. 3.—Kubanka wheat (pots 12 to 18). Set grown outside of shelter, May 9 to August 31, 1912. Photographed on July 26, 1912. Water requirement 507±13.

Fig. 4.—Emmer (pots 61 to 66), grown May 11 to August 12, 1912. Photographed on July 19, 1912. Water requirement, 428±3.

Fig. 5.—Swedish Select oats (pots 85 to 90), grown May 17 to August 23, 1912. Photographed on July 26, 1912. Water requirement, 423±5.

Fig. 6.—Kharkov wheat (pots 37 to 42), grown April 27 to August 28, 1912. Photographed on July 29, 1912. Water requirement, 365±6.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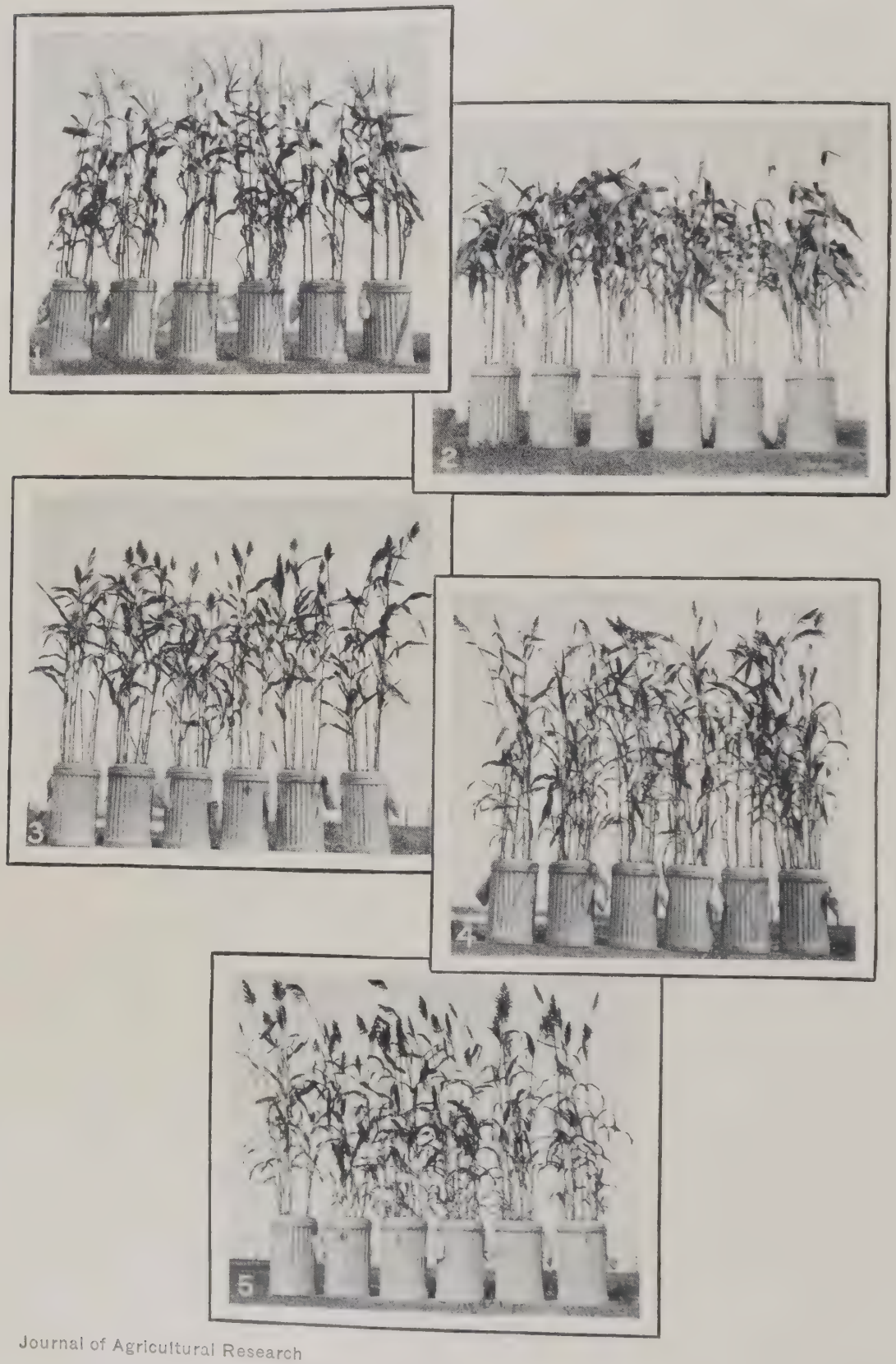
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PLATE IV

Fig. 1.—Northwestern Dent corn (pots 277 to 282), grown June 9 to September 16, 1912. Photographed on September 6, 1912. The lower leaves were picked off as they became dry and placed in bags attached to the pots, thus avoiding loss of dry matter. Water requirement, 280±10.

Fig. 2.—Hopi corn (pots 295 to 300), grown June 12 to September 26, 1912. Photographed on September 9, 1912. The lower leaves were picked off as they became dry and placed in bags attached to the pots. Water requirement, 285±7.

Fig. 3.—White durra (pots 235 to 240), grown June 9 to September 26, 1912. Photographed on September 6, 1912. The lower leaves were removed as soon as they became dry and placed in bags attached to the pots. Water requirement, 255±3.

Fig. 4.—Red Amber sorghum (pots 253 to 258), grown June 29 to September 27, 1912. Photographed on September 7, 1912. Water requirement, 237±4.

Fig. 5.—Minnesota Amber sorghum (pots 247 to 252), grown June 9 to September 26, 1912. Photographed on September 9, 1912. Water requirement, 239±2.

PLATE V

Fig. 1.—Sudan grass (pots 211 to 216). First crop, grown May 28 to July 26, 1912. Photographed on July 25, 1912. Water requirement, 312±3.

Fig. 2.—Voronezh proso (pots 199 to 204), grown June 5 to August 20, 1912. Photographed on July 29, 1912. Water requirement, 206±1.

Fig. 3.—Kursk millet (pots 205 to 210), grown June 9 to August 20, 1912. Photographed on July 30, 1912. Water requirement, 187±2.

Fig. 4.—Select Grimm alfalfa (pots 139 to 144), grown in the open, May 24 to July 27, 1912. Photographed on July 26, 1912. Water requirement, 745±22.

Fig. 5.—Select Grimm alfalfa (pots 133 to 138), grown in the shelter, May 24 to July 26, 1912. Photographed on July 26, 1912. Water requirement, 600±17.

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PLATE VI

Fig. 1.—Cowpea (pots 151 to 156), grown June 17 to August 26, 1913. Photographed on July 26, 1913. Water requirement, 571±3.

Fig. 2.—Hairy vetch (pots 181 to 186), grown May 29 to July 18, 1913. Photographed on July 17, 1913. Water requirement, 672±9.

Fig. 3.—Soy bean (pots 193 to 198), grown June 1 to August 26, 1913. Photographed on July 26, 1913. Water requirement, 690±8.

Fig. 4.—Cantaloupe (pots 325 to 330), grown June 14 to September 13, 1913. Photographed in place on July 26, 1913. Water requirement, 778±34.

Fig. 5.—Indian Flint corn (pots 253 to 258), grown June 7 to August 27, 1913. Photographed on July 26, 1913. Water requirement, 342±5.

Fig. 6.—McCormick potato (pots 121 to 126), grown June 5 to October 4, 1913. Photographed on July 26, 1913. Water requirement, 717±11.

PLATE VII

Fig. 1.—Triumph cotton in shelter (pots 163 to 168), grown May 29 to September 16, 1913. Photographed on September 15, 1913. Water requirement, 657±11.

Fig. 2.—*Boebera papposa*, grown July 25 to September 17, 1913. Photographed on September 15, 1913. Water requirement, 881±26. *Helianthus petiolaris* in background.

Fig. 3.—Rice in shelter (pots 157 to 162), grown June 12 to September 16, 1913. Photographed on September 15, 1913. Water requirement, 744±17.

Fig. 4.—General view of the shelter, showing emmer at the left and White Hull-less barley at the right. Photographed on July 3, 1912.

Fig. 5.—General view in the shelter, showing corn in the foreground. Photographed on September 6, 1912.

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HEART-ROT OF OAKS AND POPLARS CAUSED BY POLYPORUS DRYOPHILUS

By GEORGE G. HEDGCOCK, *Pathologist*, and W. H. LONG, *Forest Pathologist, Investigations in Forest Pathology, Bureau of Plant Industry*

INTRODUCTION

The oaks (*Quercus* spp.) of the United States are diseased by a number of species of fungi which attack the heartwood. Von Schrenk and Spaulding (1909)[1] briefly described some of these diseases and also a piped rot of the heartwood of oaks and chestnuts (*Castanea dentata*) the cause of which was unknown to them. In 1909, the senior writer found *Polyporus dryophilus* constantly associated with a whitish piped rot of several species of oaks in the southwestern and western United States. This rot was much like that described by Von Schrenk and Spaulding and was identical with that of specimens in oak collected by them. Later observations by the senior writer established the causal relation of *Polyporus dryophilus* to this piped rot.

The junior writer in 1913 found a second form of piped rot caused by *Polyporus pilotae* in the heart-wood of the root and basal portion of the trunks of oaks and also in chestnuts. This was identical with the rot in chestnut trees figured and collected by Von Schrenk and Spaulding.

The oaks of the southwestern and western United States are not used to any extent for lumber and timbers and are, as a rule, valuable only for fuel. This is due to the rotted condition of the heartwood in the larger and older trees. For example, the trunks of the valley oak (*Quercus lobata*),[2] which attains a large size in the valleys of central California, are usually either badly decayed or hollow and are of no value except for the poor grade of fuel they furnish. The senior writer in 1909 ascertained that *Polyporus dryophilus* was the chief cause of the deterioration of the oaks of the western United States. Meinecke (1914) reports a destructive heart-rot of oaks caused by this fungus in California and Nevada, and data by him will be cited in the section on the distribution of the fungus. In Arizona and New Mexico the oaks are diseased in the heartwood nearly as badly as in California and Oregon, and *P. dryophilus* is the common cause of decay. In these States oaks are usually small and are valuable only for fuel.

In Texas and the adjacent States of Oklahoma and Arkansas the piped rot produced by this fungus is very common, and among other

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 77.

[2] The nomenclature for trees used in this paper is that of George B. Sudworth (1898).

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species the valuable white oak (*Quercus alba*) is commonly attacked. To the east and north the fungus has been found less frequently, but it occurs in many sections.

From observations and estimates *Polyporus dryophilus* ranks with the most common heart-rotting fungi which attack the oaks. In 1912 the senior writer found aspens (*Populus tremuloides*) in Colorado attacked by this fungus. It apparently is not commonly found on this host.

PIPED ROT CAUSED BY POLYPORUS DRYOPHILUS

The whitish piped rot caused by *Polyporus dryophilus* has been found by the writers to be directly associated with the sporophores of this fungus in the following 15 species of trees: *Quercus alba*, *Q. arizonica*, *Q. californica*, *Q. digitata*, *Q. emoryii*, *Q. gambelii*, *Q. garryana*, *Q. marilandica*, *Q. minor*, *Q. prinoides*, *Q. prinus*, *Q. texana*, *Q. velutina*, *Q. virginiana*, and *Populus tremuloides*.

PIPED ROT IN THE WHITE OAK

MACROSCOPIC CHARACTERS

The first indication of the whitish piped rot in white oak is a discoloration of the heartwood, which assumes a water-soaked appearance (Pl. VIII, fig. 1). This "soak" may extend from 1 to 10 feet beyond the actually rotting region where delignification is occurring. When dry, this water-soaked heartwood becomes hazel to tawny in color. The next stage of the rot is one of delignification, which usually begins alongside of and following more or less regularly the medullary rays, thus producing a mottled appearance of the wood in radial view (Pl. VIII, figs. 2, 5, and 6). This type of the rot is very common in the medium-sized branches (6 to 12 inches in diameter) and in the early stages of the disease in the bole of the tree. In final stages the diseased wood is firm, has a white, stringy appearance (Pl. VIII, figs. 3 and 4) and consists of white cellulose strands of delignified wood fibers and other wood structures bounded by areas of apparently sound but actually slightly diseased and discolored heartwood. Cinnamon-brown areas are scattered throughout the oldest rotted wood (Pl. VIII, fig. 3). These areas are especially common and abundant in the vicinity of sporophores and along checks or openings through the sapwood. The rot immediately adjacent to a sporophore is therefore often cinnamon brown to russet in color. No cavities large enough to be seen by the naked eye are produced by this rot, but much of the white cellulose is finally absorbed, leaving minute irregular cavities in the wood.

MICROSCOPIC CHARACTERS

Delignification usually begins in the wood fibers lying next to the vessels in the spring wood and adjacent to the large medullary rays. The solvents secreted by this fungus apparently are able to delignify all of the

elements of the wood. All, or only the outer rows, of cells of the large medullary rays may be delignified, the middle lamellæ dissolved, and the completely delignified cell membranes partially absorbed.

Isolated areas between the large medullary rays may also be delignified. The cells of some of the medullary rays and of the wood parenchyma often contain starch grains even after the absorption of a portion of the inclosing cell walls. A ferruginous substance is also present in many of the cells of the small medullary rays, in the lumen of the wood fibers, and even in some of the other wood structures. Many of the vessels adjacent to each large medullary ray contain hyaline branching hyphæ 0.5 to 1μ in diameter. The association of the delignified areas with the medullary rays is readily seen in a cross section of the wood where delignification is just beginning, but later in the more advanced stages of the rot this association is not so evident when the delignification of the wood fibers has become general throughout the rotting area. The early absorption of portions of the delignified tissue prevents the formation of long continuous strands of cellulose fibers, although in a tangential view irregular white lines may be seen which consist of fragments of the delignified cells (Pl. VIII, fig. 5). In very advanced stages of the rot near the center of the tree white longitudinal lines are seen in a radial view (Pl. VIII, fig. 4). These usually consist of remnants of partially absorbed cellulose fibers bound together by strands of white mycelium, which also fill the vessels and the minute cavities left by the absorption of the delignified tissue.

PIPED ROT IN CHESTNUT OAK

The rot produced by *Polyporus dryophilus* in the chestnut oak (*Quercus prinus*) is slightly different from that in white oak. The diseased wood is hazel in color, with very narrow concentric zones of ivory-yellow cellulose. These zones are adjacent to the large spring vessels of each year and consist of the delignified wood fibers of this tissue. The large vessels in radial-longitudinal view are seen, even under a hand lens, to be filled with cobwebby strands of colorless hyphæ. It is in the tissue adjacent to such hyphæ-filled vessels where the delignification is most pronounced.

PIPED ROT IN THE WESTERN OAKS

The rot caused by *Polyporus dryophilus* in these oaks differs but little from that found in the white oak. The mottled appearance of the rot in its earlier stages is not so pronounced. In the final stage of the rot, after a very large proportion of all the elements is delignified, there is but little apparently sound heartwood. In the older rot in the center of the heartwood the white color by far exceeds the brown, of which there is very little.

PIPED ROT IN EUROPEAN OAKS

Robert Hartig (1878), in his epoch-making work on the true nature of the rots of woods, described a whitish heart-rot of the oak, which he attributed to *Polyporus dryadeus*. A careful study of Hartig's figures, and the description of the sporophore which he found associated with the white heart-rot so accurately described by him, is sufficient to convince anyone who is familiar with the true *P. dryadeus* that Hartig's fungus was not *P. dryadeus*. It is undoubtedly identical with the heart-rotting fungus known in America as *P. dryophilus* and found by the senior author to be associated with a whitish piped rot in oak. Through the kindness of Dr. Von Tubeuf the junior writer obtained a piece of the original rot (Pl. VIII, figs. 7 and 8) which Robert Hartig (1878) ascribed to *P. dryadeus*. A careful study of this specimen showed that it is identical in every respect with the rot produced by *P. dryophilus* in the white oak. There is also another European specimen (Pl. VIII, fig. 9) of this rot in oak in the Laboratory of Forest Pathology, of the Department of Agriculture, which has all the characters of the rot produced by *P. dryophilus*.

CHARACTERS OF PIPED ROT COMMON TO ALL SPECIES OF OAKS

The rots produced by *Polyporus dryophilus* in all the species of oak examined had the following characters in common: (1) A water-soaked discolored area in the first stage (Pl. VIII, fig. 1); (2) a general association of the earlier delignification with the medullary rays (Pl. VIII, figs. 5 and 6); (3) later a more general delignification of all the wood fibers (Pl. VIII, fig. 3); (4) the formation of white mycelial longitudinal lines (Pl. VIII, fig. 4); (5) the presence of cinnamon-brown areas in the older rotted wood (Pl. VIII, fig. 3). These brown patches, ranging from 2 by 4 mm. up to 10 by 35 mm. in size, consist of fragments of wood interwoven with ferruginous, thick-walled, septate hyphæ, which easily break into short pieces. The hyphæ are about 3μ thick, have many short (3 to 8μ) branches, and are mixed with various sizes of hyphæ down to 1μ or less in diameter, the smaller of which are hyaline.

HEART-ROT PRODUCED BY POLYPORUS DRYOPHILUS IN ASPEN

The description of the heart-rot which follows was made from the diseased wood of a dead aspen (*Populus tremuloides*) bearing the sporophores of *Polyporus dryophilus*.

MACROSCOPIC CHARACTERS

The general color of the diseased wood varies from a light buff to a maize yellow. In a cross section the rotted wood shows alternating concentric zones of light buff and ochraceous tawny. The light-colored zones consist of the vessels and wood fibers which have been the most vigorously attacked by the solvents of the fungus. The ochraceous-

tawny zones consist of vessels, cells of wood parenchyma, and other elements of the wood in the cells of which a ferruginous amorphous substance has been deposited. These cells are not as strongly attacked by the fungus as are those of the light zones. The rotted wood easily splits into concentric layers, the cleavage usually occurring along the boundary between the white and dark zones. In a tangential view, small, more or less isolated areas of delignified wood fibers may be seen. These delignified fibers are most abundant in the older, rotted portion. In the vicinity of the sporophores the typical cinnamon-brown areas seen in the oak are also present. The rotted wood is soft, almost silky to the touch, is very light in weight, and is easily broken into fragments between the fingers.

MICROSCOPIC CHARACTERS

The vessels in the light-colored zone have very thin walls, owing to the action of the fungus; the bordered pits are often eroded until only large irregularly shaped holes are left and the middle lamellæ of the vessels and of the wood fibers in this region are dissolved. The wood fibers and some of the adjacent cells are finally delignified and absorbed. The delignification occurs most rapidly along the boundary lines between the light-colored and dark-colored zones, along which the cleavage commonly occurs. The small amount of delignified fibers present and their rather rapid absorption prevent the formation of the large areas of white cellulose which are so common in the rot produced by this fungus in oak. In the zone of cleavage cobwebby masses of white mycelium occur which fill the vessels and the small cavities left by the absorption of the wood fibers. The medullary rays are readily attacked by the solvents of this fungus and usually have completely disappeared by the time the final stage of the rot is reached.

ENTRANCE OF THE ROT IN THE HOST

Polyporus dryophilus, so far as known to the writers, gains entrance in the wood of the host trees only through wounds in which the heartwood is exposed. The most common point of entrance is a broken or dead limb, although in the western and southwestern United States it also frequently enters through fire scars and other basal wounds.

In Arkansas and eastward, where the species of oaks differ from those in the West and Southwest, the rot caused by this fungus is apparently confined chiefly to the branches and upper portion of the trunk. This may be due to the fact that often there are one or more large dead branches in the crown of the tree, while there are very few on the lower part of the trunk. The fungus has therefore little or no opportunity to enter the bole of the tree below the crown.

When the fungus enters the stub of a broken limb, it grows downward through heartwood of the stub till it enters the trunk, when it spreads

both upward and downward through the heart of the tree. When it enters near the base of the tree, it sometimes spreads upward throughout the heart of the entire trunk. This occasionally was noted in the white oak in Arkansas, and such trees were worthless for lumber.

In Oklahoma and to the west oaks frequently have large dead branches at any point on the trunk of the tree. Through these the fungus may enter. The rot therefore is not confined as closely to the upper half of the trees as it is in the oaks of Arkansas and to the east. Probably 50 per cent of the western oaks attacked by this fungus have the rot throughout the entire trunk.

The sporophores of *Polyporus dryophilus* when growing on oak are usually found only on living trees; however, specimens have been collected growing on the boles and large branches of trees which had been cut for at least three years, and in one instance a sporophore was found growing directly on the top of an old oak stump. The fungus apparently continues to grow slowly in the infected trees after they have been cut, but rarely fruits under such conditions. There is no evidence at hand concerning the possibility of infection by *P. dryophilus* after the death of the tree.

In no instance in Arkansas has the junior writer found this fungus entering a tree through fire scars or other wounds on the butt of oaks, even where fire scars were common. The rot always originated at some point above the base of the tree, and if a tree was found in which the rot had reached the collar of the tree it came from above and not from below. All of the sporophores of this fungus found on specimens of Populus were growing on dead or dying trees. In this case the fungus is able to fruit abundantly on both living and dead trees.

This fungus on Populus seems to be truly parasitic, to some extent at least. It attacks the trunks of the trees chiefly, entering the heartwood through dead limbs after they are broken off. The trees die by either breaking off or in some cases apparently from the direct effect of the fungus, which attacks the sapwood when the disease becomes far advanced.

Several instances were found in oak where the fungus had apparently penetrated and killed small areas of the sapwood and formed its sporophores at these points.

No positive evidence was found indicative of the age of the fungus in either oaks or poplars or of its rate of growth in the infected tree. Apparently trees of all ages are susceptible to this rot, provided the branches are old enough to have formed heartwood.

SPOROPHORE OF POLYPORUS DRYOPHILUS

Polyporus dryophilus has a hard, granular, sandstone-like core, a character that is unique and not possessed by any other polypore known to the writers. The sporophore of this plant, represented by numerous specimens collected by the writers in various portions of the United

States, in every instance shows this hard granular core (Pl. IX, figs. 2 and 4) exactly as figured and described by Hartig (1878) in case of his *P. dryadeus*. This core extends back some distance into the tree in oaks; it is usually irregularly cylindrical while in the tree, but on its emergence from the tree it swells into a tuberous or spheroid mass and finally occupies the central and rear part of the sporophore (Pls. IX, fig. 2, and X, fig. 6). If the sporophore is formed from a large branch hole, it is usually of the applanate type, with a small core, but when the sporophore forms directly on the body of the tree, as it usually does, the shape is tuberous, unguliform, or even subglobular (Pl. IX, figs. 2 and 4), with the bulk of the sporophore composed of hard, granular core. This core usually has white mycelial strands (Pl. IX, fig. 4). The sporophore of *P. dryophilus*, therefore, has normally three distinct kinds of structures (Pl. X, fig. 4): (1) The hard, granular core; (2) the fibrous layer which surrounds this core except at the rear; (3) the layer of tubes on the lower surface. Specimens are often found, however, especially from the western part of the United States, in which this fibrous layer may be entirely absent between the tubes and the granular core (Pl. IX, fig. 4).

Polyporus dryophilus is known in Europe under at least five different names: *Polyporus fulvus* Fries, *P. friesii* Bresadola, and *P. corruscans* Fries for the form on oak, and *P. vulpinus* Fries and *P. rheades* Persoon for the form on poplar. The identity of *P. dryophilus* with the *P. corruscans* Fries (Pl. X, fig. 4) and with *P. rheades* Persoon is based on the specimens of these plants found in the Lloyd Herbarium at Cincinnati, Ohio. If these specimens are correctly determined, then the American plant is identical with the European plants named above. Authentic specimens of the form of *P. dryophilus* found on species of Populus were seen by the junior writer at the New York Botanical Gardens in collections from Finland and Sweden and also from Maine. In the Lloyd Herbarium at Cincinnati, Ohio, are collections under the name of *P. rheades* on *Populus tremula* from Sweden (Pl. X, fig. 3) and Denmark, and a collection from Austria on *Quercus ilex*. In the Cryptogamic Herbarium of Harvard University there is a collection on *Populus grandidentata* Michx. from New Hampshire, while in the Laboratory of Forest Pathology there is a fine collection on *Populus tremuloides* Michx. (Pl. X, figs. 1, 2, and 5) from near Steamboat Springs, Colo.

This fungus on Populus agrees in all essential characters with the form of *Polyporus dryophilus* found on oak. The sporophores are, however, somewhat smaller than those usually found on oak and approach the applanate type (Pl. X, figs. 1 and 2). The hard granular core is always present, but is formed between the sapwood and bark (Pl. X, fig. 4), as the fungus is able to rot the sapwood, as well as the heart of this host. It therefore does not have to depend on branch holes or other openings through the sapwood in order to form its sporophores as it does in the oak.

DESCRIPTION OF THE SPOROPHORE OF POLYPORUS DRYOPHILUS

Pileus thick, unequal, smooth to irregular nodulose, often convex below, unguliform (Pl. IX, fig. 5), subglobose (Pl. IX, fig. 1) or even applanate (Pl. X, fig. 1), simple or rarely subimbricate (Pl. X, figs. 2 and 5), rigid, 4 to 22 cm. broad by 3 to 13 cm. wide (measured from front to rear of sporophore) by 2.5 to 21 cm. thick (measured from pore surface to top of sporophore); surface at first densely tomentose, becoming scabrous to smooth with age; tomentum rather stiff, deciduous, short, maize yellow to ferruginous; surface of weathered sporophores after the tomentum has partially disappeared, zonate, zones several, narrow, extending entirely around the pileus near its margin (Pl. IX, fig. 3); margin in immature specimens thick, usually obtuse (Pls. IX, figs. 1 and 5, and X, figs. 1 and 2), concolorous or slightly pallid, entire or undulate; context dual, consisting of a hard granular core, surrounded except in the rear by a thin fibrous layer; core subglobose to pulvinate, 3 to 10 cm. thick, ferruginous to cinnamon brown, granular, often with white mycelial strands ramifying through it (Pl. IX, fig. 2); fibrous layer on upper surface of core a mere pellicle about 0.5 mm. thick, expanding in mature specimens into a border (Pl. IX, fig. 4) 1 to 3 cm. wide and 5 to 15 mm. thick; fibrous layer between tubes and core thin, 1 to 15 mm., usually not over 6 to 8 mm., fibrous layer zonate, concolorous; tubes slender, concolorous or slightly paler than core in some specimens, rather fragile in age, 5 mm. to 3.5 cm. long, shorter near margin of sporophore, usually about 1 cm. long; mouths regular when young, but becoming somewhat irregular and angular at maturity (Pls. IX, fig. 6, and X, fig. 8), two or three to a mm., glistening, grayish when young, becoming hazel to russet with age, edges thin; spores broadly oval, smooth, ferruginous, 4.8 to 8 by 3.4 to 6.4μ, average size 6.54 by 4.85μ when on oak (Pl. IX, fig. 6), 4.8 to 6.4 by 3.4 to 5.6μ, average size 5.82 by 4.05μ when on poplar (Pl. X, fig. 8); cystidia none; hyphæ ferruginous, 4 to 6μ. The sporophores found on oak in Arkansas and in the eastern portion of the United States often have shorter tubes (Pl. IX, fig. 4), slightly smaller spores, and a more applanate pileus than those found in the Western States (Pl. IX, fig. 2).

DISTRIBUTION OF POLYPORUS DRYOPHILUS

The rot caused by *Polyporus dryophilus* is very widely distributed in the United States, having been found in 23 States: Arizona, Arkansas, California, Colorado, Illinois, Kansas, Louisiana, Maine, Maryland, Mississippi, Missouri, Nebraska, New Hampshire, New Mexico, New York, North Carolina, Ohio, Oklahoma, Oregon, Pennsylvania, Tennessee, Texas, and Wisconsin. Authentic specimens of the fungus have also been examined from the following foreign countries: Austria, Denmark, Finland, France, Germany, and Sweden. The sporophores of the fungus are frequent and the rot caused by the fungus is exceedingly common in New Mexico, Arizona, and California.

DISTRIBUTION IN EUROPE

Polyporus dryophilus is known to occur in Europe as follows, the junior writer having examined authentic specimens:

GERMANY (?):

On *Quercus* sp. (F. P. 12404)[1].

[1] "F. P."=Forest-Pathology Investigations number.

GERMANY:

On *Quercus* sp.—ROBERT HARTIG (from Herb. Von Tubeuf); PFEIFFER (Herb. N. Y. Bot. Gard.), part of the type specimen for *Polyporus friesii;* **Berlin,** LLOYD (Herb. Lloyd).

AUSTRIA:

On *Quercus ilex.*—Travnik, REV. E. BRANDIS (No. 08864, Herb. Lloyd).

DENMARK:

On *Populus* (?) sp.—J. LIND (No. 06339, Herb. Lloyd).

FINLAND:

On *Populus* sp.—**Murtiala,** Sept., 1882 (No. 5724, Herb. N. Y. Bot. Gard.).

SWEDEN:

On *Quercus robur.*—**Stockholm,** ROMELL, Oct., 1903 (Herb. N. Y. Bot. Gard.), and a second specimen, collector unknown (Herb. N. Y. Bot. Gard.).

On *Quercus* sp.—**Upsala,** LLOYD (No. 08936, Herb. Lloyd); **Stockholm,** ROMELL (No. 08936, Herb. Lloyd).

On *Populus tremula.*—**Stockholm,** ROMELL, June 25, 1905 (Herb. N. Y. Bot. Gard.), and a second specimen, MURRILL (Herb. N. Y. Bot. Gard.); **Stockholm,** HAGELUND (No. 08985, Herb. Lloyd).

On *Populus* sp.—HAGELUND (No. 09375, Herb. Lloyd); **Stockholm,** ROMELL (No. 08414, Herb. Lloyd).

FRANCE:

On *Pinus* (?) sp.—**Fontainebleau,** P. HARIOT (No. 08880, Herb. Lloyd); this specimen from France was reported as on pine, and has spores similar in size and shape to those growing on species of Populus and a sporophore much like those found on species of Quercus.

DISTRIBUTION IN UNITED STATES

Polyporus dryophilus has been reported from and collected in the various States of this country as follows:

MAINE:

On *Populus tremuloides.*—**Piscataquis Co.,** MURRILL, in 1905 (Herb. No. 1901, N. Y. Bot. Gard.).

On *Betula* (?) sp.—Near **Moosehead Lake,** VON SCHRENK, in 1899 (Herb. N. Y. Bot. Gard.).

NEW HAMPSHIRE:

On *Populus grandidentata.*—**Chocorua,** FARLOW (?), in 1904 (Herb. W. G. Farlow).

NEW YORK:

On *Quercus alba.*—**Bronx Park,** MURRILL, in 1908 (F. P. 1416).

PENNSYLVANIA:

On *Quercus* (?) sp.—**Kittanning,** SUMSTINE 32 (Herb. N. Y. Bot. Gard.).

MARYLAND:

On *Quercus alba, Q. coccinea,* and *Q. minor.*—**Takoma Park,** HEDGCOCK, in 1910.

OHIO:

On *Quercus* (?) sp.—M. A. CURTIS, "Ex. Berkeley" (Herb. W. G. Farlow); **Preston** (?), A. P. MORGAN, in 1887 (Herb. N. Y. Bot. Gard.); **Preston, A. P. MORGAN** (0598); and **Akron,** C. D. SMITH (07556, Herb. Lloyd).

VIRGINIA:

On *Quercus prinus.*—**Elkins,** LONG, in 1913 (F. P. 12418).

On *Quercus* (?) sp.—**Falls Church,** LUTTRELL, in 1902 (Herb. N. Y. Bot. Gard.).

NORTH CAROLINA:

On *Quercus prinus.*—**Brim,** LONG.

On *Quercus velutina.*—**Jonesboro,** P. L. BUTTRICK, in 1913 (F. P. 15045).

TENNESSEE:

On *Quercus alba* and *Q. velutina*.—**Roan Mountain,** HEDGCOCK, in 1913.

MISSISSIPPI:

On *Quercus lyrata*.—**Sand Point,** HEDGCOCK, in 1908.

LOUISIANA:

On *Quercus lyrata, Q. marilandica, Q. michauxii,* and *Q. phellos*.—Near **Bogalusa,** HEDGCOCK, in 1908.

MISSOURI:

On *Quercus alba*.—**Mountain Grove,** HEDGCOCK.

On *Quercus imbricaria*.—Near **St. Louis,** J. N. GLADFELTER (No. 1214, Herb. Mo. Bot. Gard.).

On *Quercus marilandica*.—**Steelville,** SPAULDING; **Mountain Grove,** HEDGCOCK.

On *Quercus minor*.—**Webster Groves,** A. H. GRAVES, in 1909 (F. P. 1617).

On *Quercus palustris*.—**Mountain Grove,** HEDGCOCK.

ILLINOIS:

On *Quercus alba*.—Near **Plymouth,** HEDGCOCK, October, 1909.

WISCONSIN:

On *Populus* sp.—**Oakfield,** in 1903 (Herb. Univ. Wisc.).

On *Quercus macrocarpa*.—**Rockton,** L. H. PAMMEL, in 1886 (Herb. N. Y. Bot. Gard.).

NEBRASKA:

On *Quercus macrocarpa*.—Near **Nelson,** HEDGCOCK, in 1911.

OKLAHOMA:

On *Quercus alba*.—**Cache,** LONG, in 1912 (F. P. 12407).

On *Quercus marilandica*.—**Cache,** LONG, in 1912 (F. P. 12420).

On *Quercus minor*.—**Cache,** LONG, in 1912 (F. P. 12408, 12416, 12419, 12421).

On *Quercus prinoides*.—**Cache,** LONG, in 1912 (F. P. 12414).

ARKANSAS:

On *Quercus alba*.—TREAT (F. P. 12102), **Casteel** (Ozark National Forest; F. P. 12137, 12140, 12142, 12154, 12219, 12243, 12263, 12268, 12296, 12402, 12403, 12405, 12406, 12409, 12413, 12425); **Bigflat** (F. P. 12158, 12156, 12160), LONG, in 1912: **Womble** (F. P. 12413), **Cedar Glades** (F. P. 12422), LONG, in 1913; **Fayetteville** and **Farmington,** HEDGCOCK, in 1906.

On *Quercus digitata*.—**Casteel,** LONG, in 1912 (F. P. 12272).

On *Quercus minor*.—**Whiterock,** LONG, in 1912 (F. P. 12240).

On *Quercus texana*.—**Mountain View,** LONG, in 1912 (F. P. 12415).

On *Quercus velutina*.—**Casteel,** LONG, in 1912 (F. P. 12410).

TEXAS:

On *Quercus marilandica*.—Near **Boerne,** HEDGCOCK, in 1909 (F. P. 760).

On *Quercus minor*.—**Austin** (F. P. 12424) and **Denton** (F. P. 12423), LONG in 1912.

On *Quercus nigra*.—Near **Houston,** HEDGCOCK, in 1909.

On *Quercus phellos*.—Near **Houston** and near **Boerne,** HEDGCOCK, in 1909.

On *Quercus texana*.—Near **Houston** and near **Boerne,** HEDGCOCK, in 1909 (F. P. 762).

On *Quercus velutina*.—Near **Boerne,** HEDGCOCK, in 1909.

On *Quercus virginiana*.—Near **Houston** and near **Boerne,** HEDGCOCK, in 1909 (F. P. 320).

COLORADO:

On *Populus tremuloides*.—**Steamboat Springs,** HEDGCOCK, in 1912 (F. P. 3894).

On *Quercus gambelii*.—**Square Top Mountain** (San Juan National Forest; F. P. 9229); near **Mancos** (Montezuma National Forest); southeast of **Delta** (Uncompahgre National Forest); HEDGCOCK, in 1912.

NEW MEXICO:

On *Quercus arizonica*.—**Pecos,** LONG, in 1913 (F. P. 12412).

On *Quercus emoryii*.—**Mogollon Mountains** HEDGCOCK, in 1911.

On *Quercus gambelii*.—**Sandia Mountains** (Manzano National Forest), HEDGCOCK, in 1906 (F. P. 126, 230); in 1908 (F. P. 270, 551–553, 558); near **Pinos Altos** (Gila National Forest), HEDGCOCK, in 1909 (F. P. 811, 812); in **Alamo National Forest, L. L. JANES**, 1909 (F. P. 1142); **Mogollon Mountains, Bear Creek Canyon,** and **Trout Creek** (Gila National Forest), HEDGCOCK and LONG, in 1911 (F. P. 9837); **Cloudcroft, LONG**, in 1911 (F. P. 12015); **Pecos, LONG**, in 1912 (F. P. 12426).

On *Quercus oblongifolia*.—Near **Mogollon,** HEDGCOCK, in 1911.

ARIZONA:

On *Quercus arizonica*.—**ChiricahuaMountains, H. D. BURRALL**, in 1908; near **Sedona** (Coconino National Forest), HEDGCOCK, in 1910; **Santa Catalina Mountains,** HEDGCOCK, in 1911.

On *Quercus chrysolepis*.—**Sedona,** HEDGCOCK, in 1910.

On *Quercus emoryii*.—**Chiricahua Mountains,** BURRALL, in 1908; **Groom Creek** and **Crown King** (Prescott National Forest), HEDGCOCK, in 1910; **Santa Catalina Mountains,** HEDGCOCK, in 1911.

On *Quercus gambelii*.—**Groom Creek** (F. P. 4557), **Crown King** (F. P. 4877), **Sedona** (F. P. 4941), and near **Flagstaff,** HEDGCOCK, in 1910; **Santa Catalina Mountains,** HEDGCOCK and LONG, in 1911 (F. P. 9801).

On *Quercus hypoleuca*.—Near **Pinos Altos,** HEDGCOCK, in 1909.

On *Quercus oblongifolia*.—**Groom Creek** and **Crown King** (F. P. 4876) and near **Sedona,** HEDGCOCK, in 1911; **Santa Catalina Mountains,** HEDGCOCK, in 1911.

On *Quercus toumeyi*.—**Santa Catalina Mountains,** HEDGCOCK, in 1911.

CALIFORNIA:

On *Quercus californica*.—**Scott River Valley** (Klamath National Forest), HEDGCOCK, in 1909 (F. P. 1886); near **Mirror Lake** (Yosemite Park), **Clarks** (Plumas National Forest), **North Fork,** and **O'Neals** (Sierra National Forest), MEINECKE, in 1910; near **El Portal** and **Yosemite** (Yosemite Park), HEDGCOCK and MEINECKE, in 1910 (F. P. 4794); near **Kennett,** HEDGCOCK and MEINECKE, in 1911 (F. P. 9649).

On *Quercus chrysolepis*.—**El Portal** and **Yosemite,** HEDGCOCK and MEINECKE, in 1910; **North Fork** (Sierra National Forest), MEINECKE, in 1910.

On *Quercus garryana*.—**Scotts River** and **Mount Marble** (Klamath National Forest), HEDGCOCK, in 1910 (F. P. 1847).

On *Quercus lobata*.—**Stanford University,** C. F. BAKER, in 1902 (Herb. Univ. of Wisconsin); near **Chico,** HEDGCOCK, in 1909; **Dobe** and **Italian Bar** (Sierra National Forest), MEINECKE, in 1910.

On *Quercus wislizeni*.—**El Portal, Yosemite,** and near **Raymond,** HEDGCOCK, in 1910; near **Kennett,** HEDGCOCK, in 1911.

On *Quercus* sp.—**Crane Valley** (Sierra National Forest), and **El Portal,** MEINECKE, in 1910.

OREGON:

On *Quercus garryana*.—Near **Mount Hood** (Oregon National Forest) and **Rogue River Valley,** Siskyou National Forest), HEDGCOCK, in 1909 (F. P. 1717); near **Medford,** HEDGCOCK, in 1911 (F. P. 9611).

On *Quercus californica*.—**Rogue River Valley,** HEDGCOCK, in 1909.

From the foregoing data the following trees are attacked by the disease caused by *Polyporus dryophilus: Quercus alba, Q. arizonica, Q. californica, Q. chrysolepis, Q. coccinea, Q. digitata, Q. emoryii, Q. gambelii, Q. garryana, Q. hypoleuca, Q. imbricaria, Q. ilex, Q. lobata, Q. lyrata, Q. macrocarpa, Q. marilandica, Q. michauxii, Q. minor, Q. nigra, Q. oblongifolia, Q. palustris, Q. phellos, Q. prinoides, Q. prinus, Q. robur, Q. texana, Q. velutina, Q. virginiana,* and *Q. wislizeni; Populus grandidentata, P. tremula,* and *P. tremuloides; Betula* (?) sp., and *Pinus* (?) sp.

CONTROL OF THE PIPED ROT OF POLYPORUS DRYOPHILUS

The piped rot caused by *Polyporus dryophilus* is one of several important heart-rots of oaks in the United States. Suggestions made for its control will apply more or less to all of these. So long as oak trees are allowed to stand long past maturity in our wood lots and forests, heart-rots will continue to be common. The practice of leaving uncut in a lumbered area all the badly diseased trees, especially those with heart-rot, is radically wrong from the standpoint of proper forest sanitation, for this practice enables heart-rotting fungi to maintain themselves in the forest while the new generation of trees slowly develops and attains the age at which they form heartwood and thus become susceptible to the attacks of heart-rotting fungi. Trees diseased with heart-rot ought not to be left for seed trees wherever it is possible to leave healthy ones for this purpose. In hardwood forests it is often not necessary to leave seed trees, owing to the abundant sprout production, and the presence of young trees intermingled among the more mature ones.

Trees in the wood lot should be inspected annually, and all trees evidently rotted at the heart should be removed. If the trunk of a tree diseased with heart-rot is struck with an axe, it does not ring with a clear sound. The presence of the fruiting body of *Polyporus dryophilus* on a tree also is evidence of the presence of the piped rot and of the necessity of removing the tree. Sporophores on trees should be removed whenever found.

In large forested areas it is not possible to personally inspect the trees every year nor to search the forests annually for sporophores, although the present prices of good white-oak lumber nearly justify the expense necessary in a system of careful forest sanitation. It will certainly pay in lumbering tracts of oak and other valuable hardwoods to cut out all unsound or diseased trees, remove the parts that can be used, and burn the remainder. Many trees under the present methods of lumbering are left standing because they are decayed in the trunk near the butt. If cut down, these trees would be found to contain enough lumber to pay for the cost of operation. Such a procedure will lead to a better and closer utilization of our gradually decreasing supply of hardwood lumber, especially of white oak.

The destruction of all trees that are rotted in the heart in timber sales will be a step far in the direction of control for these diseases of timber. A new forest grown on areas lumbered with due regard to sanitation will be certain to be nearly free from heart-rot.

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PLATE VIII

Fig. 1.—*Quercus alba:* Crescent-shaped "soak," the initial stage of the piped rot produced by *Polyporus dryophilus;* from Arkansas.

Fig. 2.—*Quercus alba:* A radial view of the rot in a limb, showing delignification; from Arkansas.

Fig. 3.—*Quercus oblongifolia:* A radial view of rot, showing delignification; from Arizona.

Fig. 4.—*Quercus alba:* A final stage of the rot, radial view, with more complete delignification; from Arkansas.

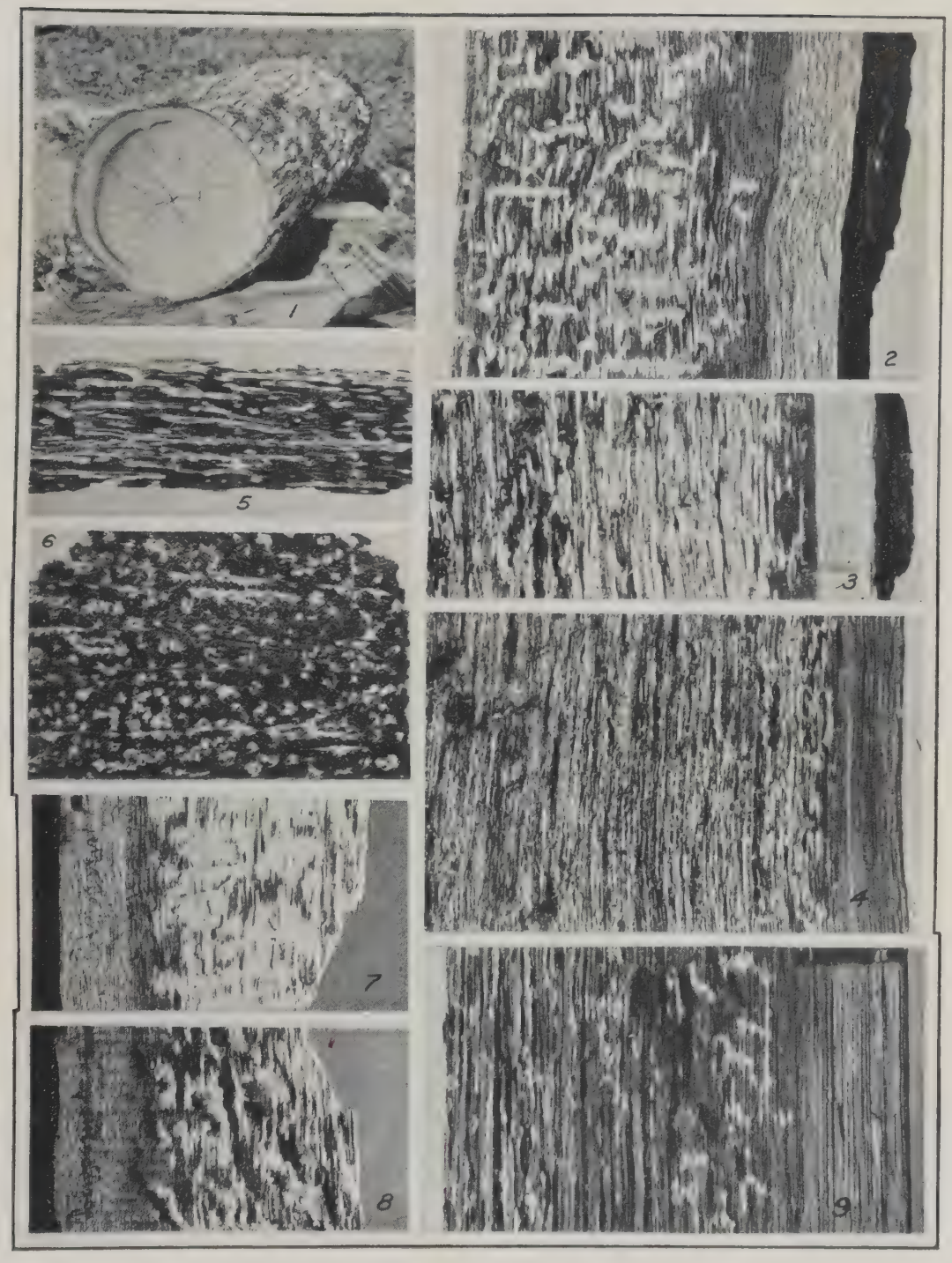
Fig. 5.—*Quercus alba:* A tangential view of the rot, showing delignification in pockets; from Arkansas.

Fig. 6.—*Quercus alba:* An end view showing a cross section from the same tree as the preceding; from Arkansas.

Fig. 7.—*Quercus* sp.: A section of oak from Von Tubeuf, sent to the junior writer as a specimen of the rot caused by *Polyporus dryadeus* in Europe.

Fig. 8.—*Quercus* sp.: The reverse side of the specimen shown in the preceding.

Fig. 9.—*Quercus* sp.: A section of oak from Europe, obtained by Von Schrenk, with a piped rot similar to that of *Polyporus dryophilus*.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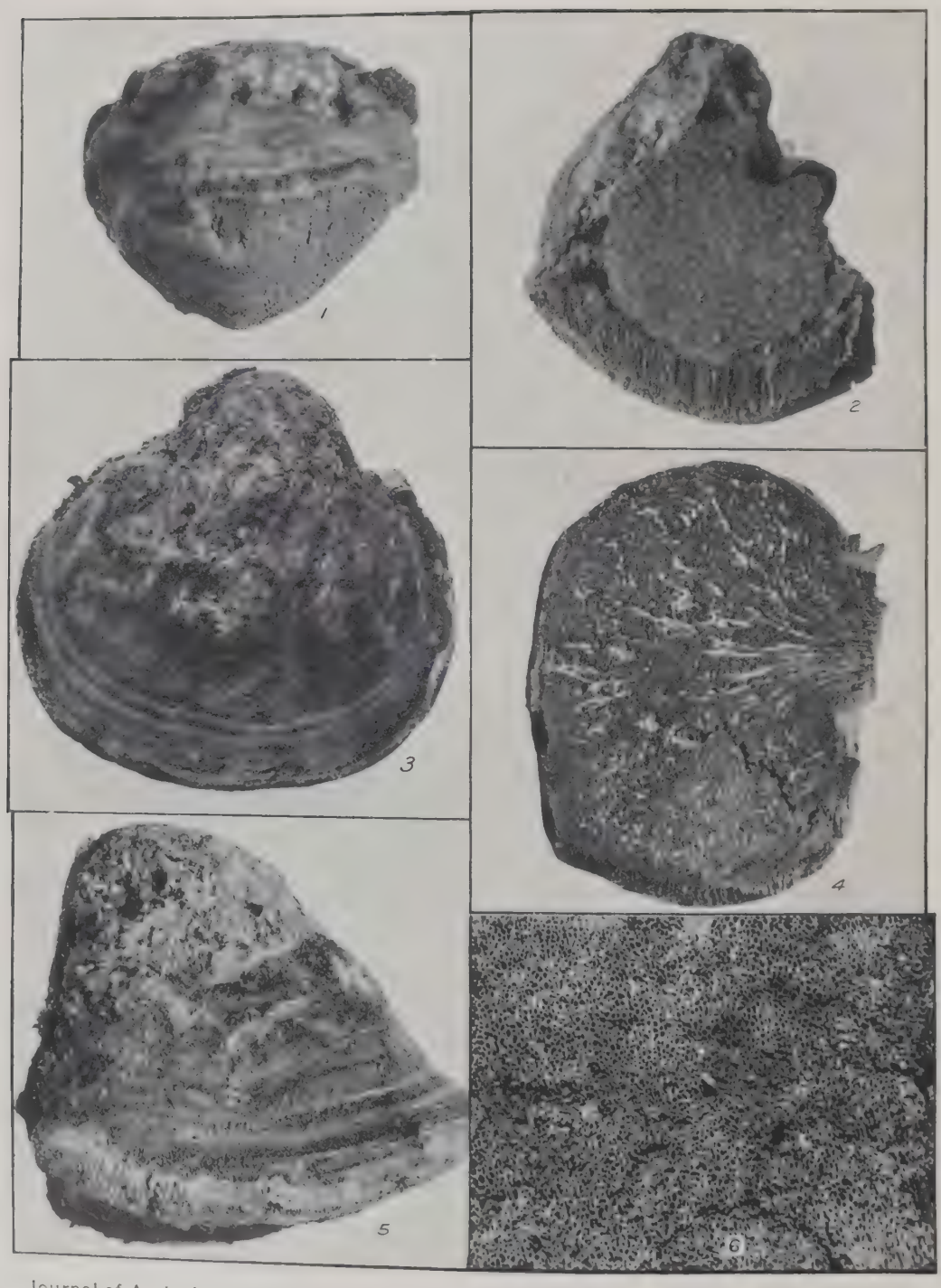
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PLATE IX

Fig. 1.—A sporophore of *Polyporus dryophilus*, tuberous form on *Quercus gambelii*; from Arizona.

Fig. 2.—Sectional view of a sporophore of *Polyporus dryophilus* on *Quercus gambelii*, showing the hard granular core with whitish mycelial strands; also the pore layer; from New Mexico.

Fig. 3.—A sporophore of *Polyporus dryophilus* on *Quercus californica*, showing the upper surface with a faint zonation; from California.

Fig. 4.—A section through a sporophore of *Polyporus dryophilus* on *Quercus garryana*, showing the structure of the hard granular core; from California.

Fig. 5.—A front view, showing the margin of the same sporophore as in figure 3, representing the ungulate form.

Fig. 6.—A view of the pore surface of an applanate sporophore of *Polyporus dryophilus* on *Quercus alba;* from Arkansas.

PLATE X

Fig. 1.—A sporophore of *Polyporus dryophilus*, front view showing the margin, on *Populus tremuloides;* from Colorado.

Fig. 2.—A second sporophore from the same tree as figure 1, showing an imbricated form.

Fig. 3.—A view of the upper surface of a sporophore of *Polyporus rheades* on *Populus tremula;* from Stockholm, Sweden.

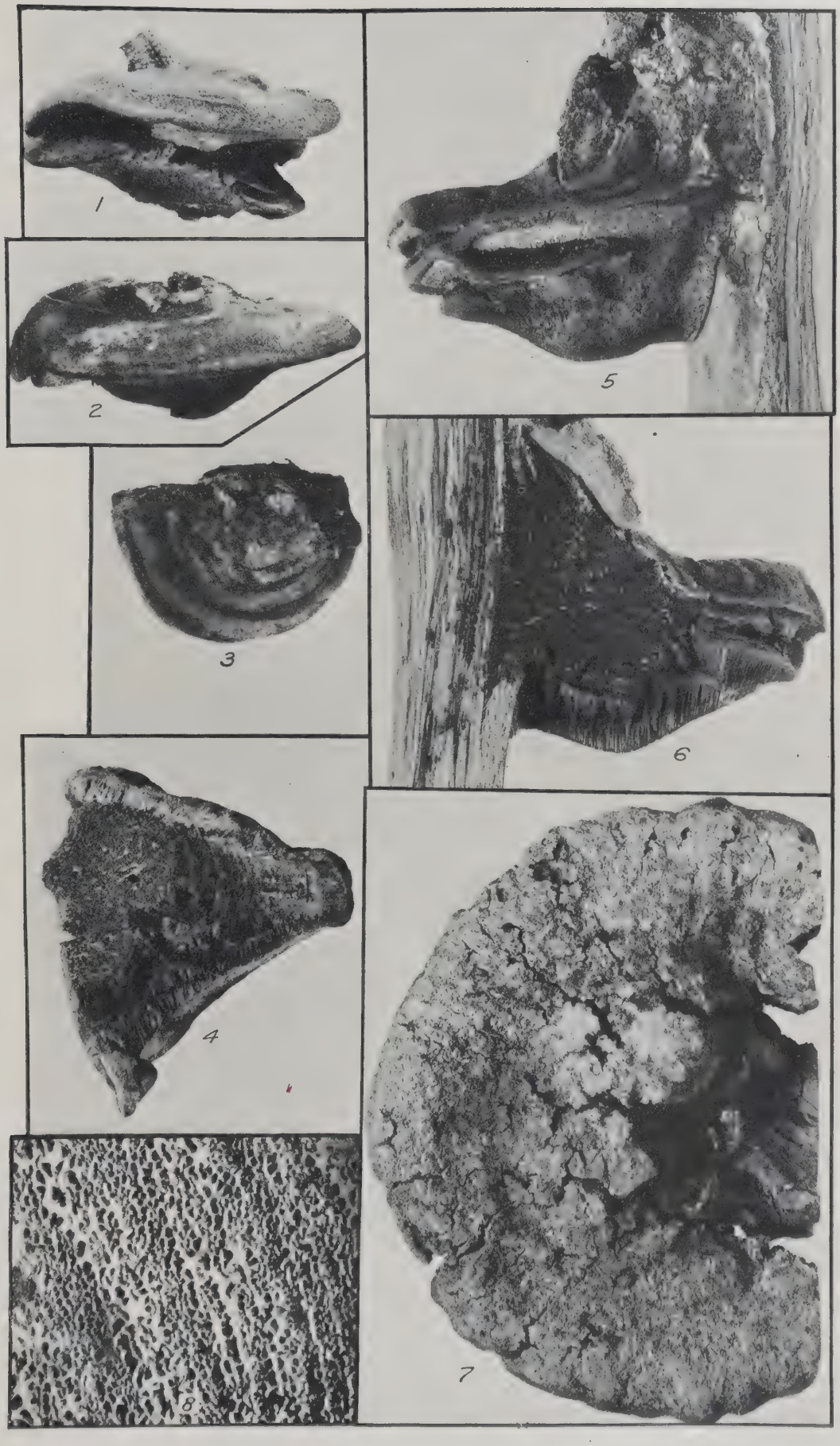
Fig. 4.—A sectional view of a sporophore of *Polyporus corruscans* on Quercus; from Upsala, Sweden.

Fig. 5.—A side view of an imbricate sporophore of *Polyporus dryophilus*, applanate form on *Populus tremuloides;* from Colorado.

Fig. 6.—A sectional view of the same sporophore as in the preceding figure, showing the hard granular core and whitish mycelial strands.

Fig. 7.—A view of the upper surface of an applanate sporophore of *Polyporus dryophilus* on *Quercus alba;* from Arkansas.

Fig. 8.—The pore surface of a sporophore of *Polyporus dryophilus* on *Populus tremuloides;* from Colorado.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PRELIMINARY AND MINOR PAPERS

DECOMPOSITION OF SOIL CARBONATES

By W. H. MacIntire,

Soil Chemist, Tennessee Agricultural Experiment Station

Investigations recently conducted at the University of Tennessee Agricultural Experiment Station have led to the discovery that the composition of soils is such as to make inhibitory any long-continued occurrence therein of magnesium carbonate, and the conclusion has been drawn that magnesium carbonate does not exist as a solid mineral in our humid soils.

The research has demonstrated that the affinity of magnesia for silica s such that soils long since alkaline from excessive treatments of calcium carbonate are able to dissipate the CO_2 of magnesium carbonate under sterile, moist conditions. It has been further demonstrated that the affinity of magnesia for silica is so great that precipitated magnesium carbonate is extensively decomposed by pure SiO_2 and also by the closely allied compound titanium oxid, which occurs almost universally to an appreciable extent in soils.

Analyses to determine residual carbonates at the end of one year established the fact that precipitated magnesium carbonate in amounts chemically equivalent to 16,070 pounds of $CaCO_3$ per acre in excess of the quantity indicated by the Veitch method as necessary to correct acidity had been entirely decomposed by each of three distinct types of unleached soil. A loam, a sandy loam, and a silty loam were used in the study. In substantiating the work the loam soil was subjected to eight check treatments in field rim experiments. In each of these eight instances precipitated $MgCO_3$ equivalent to over 15 tons of a good grade of limestone per 2,000,000 pounds of soil had entirely disappeared at the end of eight weeks, when the first analyses were made for residual carbonates. No drainage took place during this 8-week interval.

Comparisons between residual carbonates from limestone and dolomite treatments showed at the end of 9 months' exposure to weather 22,000 pounds of $CaCO_3$ per 2,000,000 pounds of soil for the limestone treatment as compared to 11,000 pounds of $CaCO_3$ as a residue from the dolomite.

The investigations have also determined that the absence of carbonates subsequent to applications of magnesium oxid has been erroneously attributed to persistent causticity of the magnesia, which is shown to be very readily converted to the carbonate, while this in turn is decomposed by siliceous substances.

The use of $CaCO_3$ as a check has shown that the affinity of lime for silica is far greater than has been supposed, and the work has demonstrated that the lime-silica reaction in soils is an important factor in the conservation of lime applied in practice. While the lime-silica reaction

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does not approach the magnesia-silica reaction in rapidity, it is shown by field data that the lime-silica reaction continues long after the attaining of alkalinity and that the reaction is extensive.

Toxicity due to excessive treatments of magnesium carbonate after its conversion to silicates was demonstrated by plant growth.

The progress of the work of the writer and associates is reported in detail in Tennessee Experiment Station Bulletin 107.

A FUNGOUS DISEASE OF HEMP

By VERA K. CHARLES, *Assistant Mycologist*, and ANNA E. JENKINS, *Scientific Assistant*, *Office of Pathological Collections and Inspection Work, Bureau of Plant Industry*

In September, 1913, the attention of the Office of Pathological Collections and Inspection Work was called to a fungous disease which had attacked a variety of hemp (*Cannabis sativa*) grown for experimental purposes by Mr. L. H. Dewey, Botanist in Charge of Fiber-Plant Investigations. Although the disease did not make its appearance until the plants were almost full grown, it was very rapid in its action, only about two weeks having elapsed between the time that the disease was first noted and the death of many of the plants. One of the early symptoms of the disease was the wilting and drooping of the leaves. The foliage turned brown and finally died, but remained attached to the plant longer than in the normal condition. In nearly all instances the fungus first attacked the outer ends of some of the upper, though rarely the highest, branches of the plant. In some cases the branches above and below the diseased area remained uninjured for some time. It was observed that the disease spread more above than below, but that the affection of the plant became general in about two weeks. Although the disease appeared to attack the outer ends of the branches first, the main stem below the base of the diseased branch became bleached and afterwards darkened by the formation of the perithecia of the fungus (Pl. XI).[1]

The hemp was grown from seed originally introduced from China, having been grown for experimental purposes during a period of 10 years. Its cultivation had been generally successful, and until the season of 1913 no difficulty had been experienced from fungus attack.

All of the plants in the plots in which the disease was most serious were from the seed of one single selected plant, the third best of the crop of 1912. This plant showed no evidence of disease and was remarkable for its purple-colored foliage. Selections had been continuous for 10 generations without any admixture of other strains. Three or four plants of this plot which were especially precocious were marked as soon as it was observed that they were pistillate, and each one of these plants was attacked by the disease. So general was the attack that among the 135 pistillate plants of this plot 128 were destroyed by the fungus, representing a loss of about 95 per cent of this plot. Later the disease appeared in a larger plot of 320 and in less than four weeks 66⅔ per cent of the plants had been attacked.

A microscopic examination of the first diseased material collected on September 12, 1913, revealed the presence of small, black pycnidia, containing minute, hyaline spores on branched conidiophores. These characters, together with the absence of stroma, placed the fungus in the genus Dendrophoma. (Fig. 1, *E* and *F*.) This appears to be the first occurrence of the fungus in America. A second examination of the diseased hemp about three weeks later showed pycnidia containing spores

[1] Most of the field observations were made by Mr. L. H. Dewey, who mentions the occurrence of this disease in an article entitled "Hemp" in the Yearbook, U. S. Dept. of Agriculture, for 1913, p. 283–346, fig. 17–21, pl. 40–46. 1914.

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characteristic of the genus Macrophoma (fig. 1, *D*). At the same date an immature ascomycete was observed on material which had been allowed to remain on the ground. The final collection on November 3 showed an ascomycetous fungus present in large amounts on the hemp which had been spread for dewretting, while the two other spore forms were absent, or present only in negligible amounts, having matured before the development of the ascospores. The asci were borne in perithecia similar in appearance to the pycnidia of the two other forms (fig. 1, *B*).

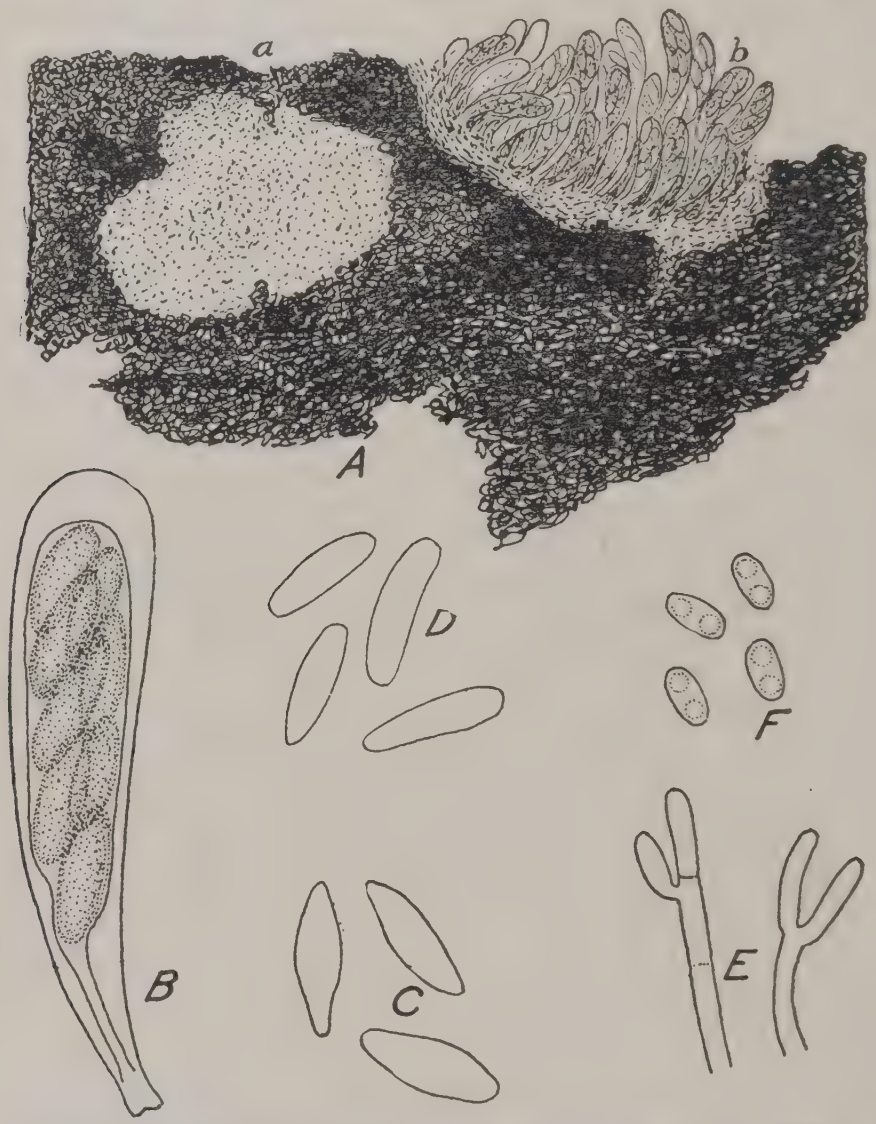

FIG. 1.—Microscopic characters of the hemp fungus *Botryosphaeria marconii*. *A*, Sketch of a section of stroma from culture, showing developing perithecia: *a*, microconidial stage, *b*, ascosporic stage, × 840. *B*, An ascus with ascospores, × 840. *C*, Ascospores, × 840. *D*, Macroconidia, × 840. *E*, Conidiophores of the Dendrophoma stage, × 1920. *F*, Microconidia, × 1920. (Drawing by J. Marion Shull.)

The spores were hyaline to slightly colored, nonseptate, and fusoid (fig. 1, *C*). A probable connection between these three forms suggested itself to the authors, and cultures were started to prove, if possible, that these stages are different phases in the life history of one fungus. The spores of the Dendrophoma form are designated as microconidia and those of the Macrophoma stage as macroconidia.

Cultures were made on various media, but as the fungus developed luxuriantly and rapidly upon corn meal, that medium was adopted for

the cultural work. The fungus developed in the same sequence as in nature, the Dendrophoma stage appearing first, regardless as to whether the cultures were made from microconidia, macroconidia, or ascospores. Sections of the pycnidia made at a later date demonstrated that the development of the macroconidia followed the microconidia in the same pycnidium. In sections made at a still later date asci were found developing in the same locule with the mature macroconidia. The three spore forms of the fungus as developed in culture agreed perfectly in character with those found in nature. The variations observed in size and shape of macroconidia and shape of the asci were also exhibited by the fungus in nature. The one notable difference, however, was in the stronger development of stroma in the cultures. Since the Dendrophoma spores and the Macrophoma spores developed in the same pycnidia, the macroconidia and ascospores in the same perithecia, and all three forms in the same stroma, it is definitely proved that these three forms represent the different stages in the life history of one fungus (fig. 1, *A*).

From the critical microscopical study of the Dendrophoma stage of this fungus in nature and in culture it is shown to be morphologically identical with a specimen of *Dendrophoma marconii* described by Cavara in Italy in 1887.[1] No stroma is produced as the fungus occurs on the host, although a well-developed stroma is produced in culture. This stromatic development is suggestive of the genus Dothiorella, but it is not a constant character, and as the fungus agrees so closely with Cavara's description of Dendrophoma on hemp,[2] the authors consider these two forms to be identical.

During the course of the microscopic study of Dr. Cavara's material a second type of spore was found which corresponded exactly with the macrospores discussed in this paper. No mention of these was made in Dr. Cavara's paper, however, and the writers were unable to determine whether or not they had been observed by him.

Among the few fungi described on hemp and related genera no species were found possessing the characters of the perfect stage of the fungus here discussed. In 1831 a fungus was observed by Schweinitz on hemp, and was called by him *Sphaeria cannabis* Schw.[3] This species is of historical interest only, for the description is too meager to be of any taxonomic value. The characters of the ascosporic stage place the fungus in the genus *Botryosphaeria* as defined by Saccardo.[4] As the imperfect stage of this fungus is considered identical with the first described form, *Dendrophoma marconii* Cav., the specific name is retained and the fungus is designated *Botryosphaeria marconii* (Cav.) Charles and Jenkins.

Botryosphaeria marconii (Cav.) Charles and Jenkins.

Perithecia globose, perforate, diseased area pale olive buff to gray, 140 to 160 μ in diameter; basidia bearing microconidia mostly dichotomously branched, septate, hyaline; microconidia polymorphic, ovate, elliptical, or terete, continuous, hyaline, 4 to 5½ by 1½ to 2 μ; macroconidia fusiform or ellipsoid, continuous, hyaline to glaucous, 16 to 18 by 5 to 6 μ; basidia of macroconidia slender, generally 12 to 15 μ in length; asci clavate, 8-spored, 80 to 90 by 13 to 15 μ; paraphyses filiform; spores fusoid, hyaline to pale light grape green, 16 to 18 by 7 to 8 μ. Microconidia, macroconidia, and asci produced in the same perithecium. On *Cannabis sativa*.

[1] Briosi, Giovanni, and Cavara, Fridiano. I Funghi Parassiti delle Piante Coltivate od Utili. no 20. Pavia, 1887. *Exsiccatæ*.

[2] Cavara, Fridiano. Appunti di patologia vegetale (alcuni funghi parassiti di piante coltivate.) *In* Atti Ist. Bot. Univ. Pavia, [s. 2], v. 1, p. 426. 1888.

[3] Schweinitz, L. D. von. Synopsis fungorum in America boreali media degentium secundum observationes. *In* Trans. Amer. Phil. Soc., n. s., v. 4, p. 222, no. 1741. 1634.
Saccardo, P. A. Sylloge Fungorum . . . v. 2, p. 432. Patavii, 1883.

[4] Saccardo, P. A. Sylloge Fungorum . . . v. 1, p. 456. Patavii, 1882.

In view of the serious nature of the disease and its sudden appearance in America it has seemed best to present this preliminary paper. The true parasitic nature of the fungus was evident from its effect on the growing plants, but its parasitism was further demonstrated by the successful isolation of the fungus from the interior tissue of thoroughly disinfected stems. Owing to limited time and opportunity for extensive field observations, many questions relating to the pathological phase of the subject remain unsolved. Problems pertaining to the method of infection by the fungus, its manner of dissemination, and control measures for the disease are still subjects of investigation by the Office of Pathological Collections and Inspection Work.

PLATE XI

A hemp plant, showing upper branches attacked by the fungus *Botryosphaeria marconii*.

A MORE ACCURATE METHOD OF COMPARING FIRST-GENERATION MAIZE HYBRIDS WITH THEIR PARENTS

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INTRODUCTION

That the crossing of two distinct varieties of maize usually results in an increase of vigor and larger yields in the first, or F_1, generation has come to be generally recognized. The amount of the increase, however, varies greatly in different hybrids, and in many cases the increase is not large enough to be determined by ordinary experimental methods, if it exists at all.

So far as known, no case has been reported where a decrease below the mean yield of the parents has been adequately demonstrated. It is highly desirable to know the conditions under which significant increases occur, but thus far little light has been thrown on this important point. If really incompatible varieties exist, a study of their behavior in hybrid combinations should afford a favorable opportunity to learn something regarding the conditions necessary for large increases. One serious obstacle to learning the factors involved in the increased yields of first-generation hybrids is the difficulty of accurately comparing the vigor and yield of a hybrid with that of the parent varieties.

Hybrids in maize are made either by hand pollination between individual plants or by planting in alternate rows the varieties to be hybridized and removing the tassels from all the plants of one of the varieties.

The customary method of comparing the behavior of a hybrid with its parents is to plant the hybrid seed in rows or blocks alternating with similar areas planted with the seed of the parents. If the series is repeated a sufficient number of times, reliable averages may be obtained, but in actual practice the number of repetitions is usually limited by lack of seed or space.[1]

In making a comparison between a hybrid and its parents where the hybrid is made by planting the varieties in alternate rows, the question arises as to what seed will best represent the parents. If the original seed of the parent varieties is used, it will be one year older than the hybrid seed, and the uncertain element of deterioration with age is introduced.[2] By saving the seed from the plants used as a source of pollen in making the hybrid, fresh seed of the male parent can be secured, but if fresh seed of the female parent is obtained, it must be grown at some distance from the place where the hybrid is made. To use seed grown under different conditions introduces an element of uncertainty that

[1] In experiments with maize extending over a number of years in many different localities, we have found that with rows 100 feet long and the series repeated 10 times, it has seldom been possible to detect with assurance differences in yield of less than 10 per cent.

[2] For a discussion of this point, see Hartley, C. P., Brown, E. B., Kyle, C. H., and Zook, L. L. Cross-breeding corn, U. S. Dept. Agr., Bur. Plant Indus. Bul. 218, p. 13–16, 1912. One and two-year-old seed of selection No. 119a, the variety used as male parent in the Maryland experiments, occurred side by side 42 times. The average superiority of the new seed was 7 ± 1.3 per cent.

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may be as serious as that incurred by the use of old seed. If the conditions of growth where the pure seed is produced are more favorable than those under which the hybrid seed is produced, natural selection will be less rigorous. There is also the possibility of direct effect of environment on the yielding power of the seed and the possibility of new-place effect.

A further disturbing factor lies in the differences between the individual plants that produce the hybrid seed and those producing the pure seed which is to represent the parental varieties. When seed from a large number of plants is used, these differences tend to counterbalance each other and give an average of value for practical purposes, but information which might extend our knowledge regarding the nature and causes of the increase may be completely obscured by this method of averaging.

Some of these difficulties can be avoided if the hybrid seed is obtained by hand pollination. By this means seed of both parent varieties of the same age as the hybrid seed and grown under similar conditions can be secured. Inaccuracies due to diversity among individual plants will, however, be increased, since the number of plants involved will necessarily be smaller, and, as before, differences in the behavior of individual crosses which might throw light on the nature of the increases will be masked if conclusions are based upon averages. To avoid this last difficulty, the individual hybrid ears may be kept separate and an ear-to-row method of making comparisons with the parents may be applied.

Differences in the breeding value of individuals are now appreciated in the breeding of pure strains and have led to the adoption of the method of separating the offspring of individuals into progeny rows. The results here reported show a diversity among the hybrid ears that result from crossing different plants of the same parent varieties that is even greater than that usually found between pure seed ears of a single variety, and the evidence indicates that individual diversity in hybrids will be found as important as in pure varieties.

In comparing an individual maize hybrid with its parents account must be taken of the fact that to behave normally maize must be cross-pollinated, and to secure cross-pollinated seed of the parent varieties two plants of each variety must be used, but only one plant of each variety can be represented in an individual hybrid ear. To avoid in some measure these sources of inaccuracy the method followed in the experiments here described is suggested.

DESCRIPTION OF METHOD

To compare the behavior of two varieties, which may be called A and B, with that of a hybrid between them, two plants were selected in each variety, A1 and A2 in the one variety and B1 and B2 in the other variety. The following hand pollinations were made: A1 × A2, A2 × B1, B1 × B2, and B2 × A1. The result is two hybrid ears and one cross-pollinated ear of each variety. It is believed that the mean yield produced by seed from the two hybrid ears compared with the mean yield produced by seed from the two pure seed ears gives a fair measure of the effects of hybridization. By making two hybrids involving all the plants used in producing the pure seed ears individual differences that affect the yielding power of the pure seed ears are similarly represented in the hybrids. Thus, in both the

parents and the hybrids the average yield represents the mean yielding power of the four parent plants, the only difference being the way in which the individuals are combined.

To secure the most accurate comparison of the yield of the four ears, one seed from each of the ears was planted in each hill. The different kinds were identified by their relative position in the hill. To place the seeds accurately, a board 4 inches square was provided with a small, pointed peg 2 inches long at each corner. These pegs were forced into the soil at each hill, making four holes, one for each of the four kinds, only one seed being planted in a hole. The board was always placed with two sides of the board parallel to the row. It was necessary to exercise extreme care in dropping the seeds to avoid changing the position of the kinds. The best way to obviate mistakes of this kind is to make all the holes of a row in advance and to go down the row with one kind of seed at a time.

At harvest time the seed produced by each plant was weighed and recorded separately. All hills that lacked one or more plants were excluded and the comparison confined to hills in which all four kinds were represented. The method of handling the yields was to determine the mean yield of the four kinds in each hill and to state the yield of each of the four plants as a percentage of the mean of the hill in which it grew. The percentage standing of each kind in all the hills was then averaged to secure the final expression of the relative behavior of the four kinds.

This method of comparison is similar to the ingenious plan originated by C. H. Kyle,[1] for use in ear-to-row breeding. Kyle's method is to plant each of the ears to be tested in a separate row and in each hill to plant one seed of a standard, or check, ear with which all ears are compared. Since comparative and not absolute yields are desired in the study of hybrids and with only four kinds to compare, the introduction of a check in the present experiment would have increased the space occupied by the experiment without lessening the experimental error.

APPLICATIONS OF METHOD

The hybrid tested by this method was a cross between the Egyptian, a white sweet corn, and the Voorhees Red, a related sweet variety with red aleurone.[2] The two hybrids secured in accordance with the foregoing method were designated Ph96 and Ph97. The use of the Voorhees Red variety as one of the parents made the comparison unusually difficult. This variety produces a considerable percentage of albino seedlings, and since no albino seedlings reach maturity, the result was a large number of hills with less than the full complement of plants. Eighty-four hills were planted, but only fifty-eight matured plants of all four kinds.

The comparative yield of the four kinds is given in Table I. To illustrate the meaning of these determinations, let us take the yield of the Egyptian variety. The number 112.8 indicates that the yield of 58 Egyptian plants averaged 112.8 per cent of the mean yield per plant of all four kinds—that is, 12.8 per cent above the mean.

[1] Kyle, C. H. Directions to cooperative corn breeders. U. S. Dept. Agr., Bur. Plant Indus., B. P. I.—564, 10 p., 1910.

[2] The strain of Egyptian corn used in this experiment was from commercial seed secured from J. M. Thorburn & Co. in 1911. The original source of the Voorhees Red was an ear kindly supplied by Prof. Byron D. Halsted, of the New Jersey State Agricultural Experiment Station, in 1907.

The mean of the two parents is 84.2±3.0 per cent of the general yield. The mean of the two hybrids is 115.9±3.3 per cent. The mean yield of the hybrids is thus 31.7±4.5 per cent higher than the mean of the parents, and this increase is ascribed to the effects of crossing.

TABLE I.—*Yield and height of the Egyptian and Voorhees Red varieties of sweet corn and two hybrids between them*

[Determinations expressed as average percentages of the mean of the four kinds.]

Variety of corn.	Yield.	Height.
	Per cent.	*Per cent.*
Egyptian	112.8±4.6	111.3±1.0
Voorhees Red	55.6±4.0	84.0± .9
Hybrid Ph96	89.0±5.1	100.0±1.2
Hybrid Ph97	142.8±4.3	103.6±1.1

A striking feature of the results obtained is the difference between the yield of the two hybrid ears, which amounts to 53.8±6.7 per cent. Had the ear Ph96 alone been taken as representing a hybrid between these varieties, the hybrid would have exceeded the average of the parents by only 4.8 per cent, a difference upon which no reliance could be placed. If, on the other hand, the ear Ph97 had been taken, the difference in favor of the hybrid would have appeared as 58.6 per cent.

The relative height of the four kinds was determined in the same manner as the yield—that is, the height of each plant was compared with the mean height of all the plants of the hill in which it grew, the latter being taken as 100. The average heights expressed in this way are given in column 2, Table I.

The average height of the parents is 97.6±0.7 per cent of the general mean. That of the hybrids is 101.8±0.8 per cent. The difference is 4.2±1.1 per cent. There is, then, a distinct increase in the height of plants as a result of crossing, but the increase is much less than the increase in yield, and the difference between the two hybrids is much less than was the case with the yield.

It has usually been found that the increase that follows crossing affects the vegetative characters even more than the reproductive. If height be taken as an index of vegetative vigor, the reverse would seem to be true in the present cross.

Increased vegetative vigor may have resulted in an increase of the branches rather than of the main stalk. To definitely settle this point, it would have been necessary to weigh or measure all of the suckers. This was not done, but the number of suckers was recorded for each of the kinds, and the difference, though small, indicates that a part of the increased vegetative vigor of the hybrids was expressed in the production of suckers. A total of 18 suckers was produced in the two pure-seed rows, while 35 were produced in the two hybrid rows. The association between vegetative vigor and yield is further shown by the fact that the hybrid Ph97 exceeded the hybrid Ph96 both in yield and in the production of suckers. It should be borne in mind, however, that an increased yield and an increased production of branches may not always be thus associated. It is to be expected that under some conditions excessive

branching may result in a decreased yield. Hence, if some hybrids show reduced yields, this fact alone should not be taken as proving an exception to the general rule that the first generation of a hybrid shows increased vigor.

The method of comparison here used brings the plants into close competition, and it may be urged that the differences between the kinds are as a result unduly accentuated. With a view to detecting a possible effect of competition, the yield of the plants in hills with four plants was compared with plants of the same varieties in hills with less than four plants.

In P9, P19, and Ph97 the yield per plant was slightly higher in the 4-plant hills than in the 3-plant hills. The differences were, however, insignificant. In Ph96 the yield of the plants from the 3-plant hills exceeded that from the 4-plant hills by 67 grams per plant. The number of 3-plant hills was so small, however, that little confidence should be placed in the difference, which was but three times the probable error.

An attempt was made to secure a more accurate comparison by correcting for the differences in the yield of the different kinds, thus making it possible to compare the yield per plant of all the 4-plant hills with that of all the 3-plant hills. The average yield per plant in the 4-plant hills was 211 ± 7 grams. The average yield of the 3-plant hills was 227 ± 10. The difference of 16 ± 12 grams is therefore not significant.

With such a large experimental error it is of course not impossible that the crowding of the plants has a tendency to reduce the yield, but if so the difference is too small to be measured by the means employed, If crowding operated to accentuate differences, it might also be expected to retard the date of flowering. The average number of days to flowering was, however, the same in the 4-plant hills and in the hills with less than four plants, being 72.4 days in both. Thus there is no evidence that the growing of the four kinds close together affects the relative yield of the kinds, and when ample space is provided between the hills, viz, 4 by 5 feet, as in this experiment, it is believed that this source of inaccuracy is insignificant.

The conditions of the experiment here reported constitute a severe test of the method of comparison by individual hills. The kinds tested were very dissimilar, while the soil of the experiment was unusually uniform. The gain in accuracy secured by using the hill as the unit of comparison, instead of averaging the yield of all the plants of a kind, may be measured by a comparison of the standard deviations or the coefficient of variability observed when the yields are compared by the two methods.

When the yield of each plant was compared with the average of all the plants of the same kind, the coefficient of variability was 5.42 ± 0.17. When the yield of each plant was compared with mean yield of the hill in which it grew the coefficient of variability was 5.05 ± 0.13. There is, thus, a slight gain in accuracy, notwithstanding the exceptional uniformity of the soil where the experiment was tried. With less uniform soil conditions the advantages secured by making the comparison on the basis of individual hills would increase.

The dates when the first staminate flowers opened and when the first silks appeared were recorded for all the plants. The average number of days from planting to flowering is shown in Table II.

TABLE II.—*Average time from planting to flowering of varieties of maize*

Variety.	Number of days to first pollen.	Number of days to first silks.
Egyptian	73.0±0.1	73.9±0.3
Voorhees Red	72.1± .2	77.1± .3
Hybrid Ph96	72.0± .2	73.5± .4
Hybrid Ph97	72.5± .2	73.6± .4

With respect to the appearance of the staminate flowers the only significant difference is the slightly later flowering of the Egyptian variety. With respect to the appearance of silks, the Voorhees Red, the low-yielding variety, was distinctly later. The average time between the opening of staminate flowers and the appearance of silks was less than one day in the Egyptian and five days in the Voorhees Red variety. Both hybrids were intermediate, with 1.5 and 1.1 days, respectively, between the average time of the appearance of pollen and silks.

A further comparison of the hybrids with their parents with respect to minor characters brings to light a number of striking differences. A comparison of the characters measured is made in Table III.

TABLE III.—*Comparison of minor characters of maize hybrids with their parents*

Character.	Egyptian maize.	Voorhees Red maize.	Hybrid Ph96.	Hybrid Ph97.	Average of parents.	Average of hybrids.
Height.....cm..	206 ±1.6	157 ±2.6	185 ±3.4	193 ±2.9	182 ±1.5	189 ±2.2
Number of suckers...	.21± .04	.11± .02	.14± .03	.48± .08	.160± .022	.312± .041
Total number of leaves.....	17.4 ± .13	15.8 ± .12	16.4 ± .13	16.8 ± .14	16.6 ± .08	16.6 ±1.0
Exsertion of tassel [a] .....cm..	4.3 ± .3	4.9 ± .3	5.2 ± .2	3.5 ± .3	4.6 ± .20	4.4 ± .17
Length of axis of tassel [b].....cm..	12.7 ± .3	16.6 ± .3	16.4 ± .3	14.1 ± .3	14.1 ± .19	15.2 ± .20
Length of central spike [c].....cm..	28.1 ± .8	23.2 ± .8	24.5 ±1	29.7 ±1.1	25.6 ± .58	27.1 ± .74
Number of primary branches in tassel..	14.4 ± .25	19.5 ± .32	20.1 ± .35	15.4 ± .17	16.9 ± .21	17.7 ± .22
Number of secondary branches in tassel..	5 ± .1	6.2 ± .2	7.2 ± .3	4.7 ± .2	5.6 ± .25	5.9 ± .18
Length of longest leaf.	59.3 ± .5	84 ± .8	89.6 ±1	92.9 ± .7	86.6 ± .47	91.2 ± .62
Number of nodes above longest leaf..	5.6 ±1.0	4.5 ±1	4.9 ±1.2	5.1 ±1.0	5.1 ± .7	5 ± .78
Number of nodes above ear.....	5.3 ± .8	4.9 ± .4	4.7 ± .6	4.9 ± .6	5.1 ± .36	4.8 ± .41

[a] Measured from the top of the uppermost leaf sheath to the lowest tassel branch.
[b] Measured along the axis from the insertion of the first to the insertion of the last primary tassel branch.
[c] Measured from insertion of last tassel branch to tip of tassel.

In all of the characters measured, with the exception of "Number of nodes above the longest leaf" and "Number of nodes above the ear," there was a measurable difference between the two parents. In the "Number of suckers" and in the four tassel measurements there was also a significant difference between the two hybrids. The mean of the hybrids shows a close approximation to the mean of the parents in the total number of leaves, exsertion of tassel, length of the central spike, number of secondary branches, and number of nodes above the ear and the longest leaf. The characters in which the hybrid exceeds the

parents are for the most part those more closely associated with vigor—viz, height, number of suckers, and length of leaf. The differences between the two hybrids are such that without exception Ph96 stands closer to the Voorhees Red variety and Ph97 closer to the Egyptian variety. It is probably a coincidence that in both hybrids the resemblance is to the female parent.

CONCLUSIONS

So large a proportion of first-generation maize hybrids have been found to give increased yields and the increase is frequently of such magnitude that the utilization of this factor of productiveness becomes a practical question. It is therefore highly desirable to understand the reasons why some crosses give favorable results and others give little or no increase over the yield of the parents. A necessary step in this direction is to develop a reliable method of measuring the effect of crossing, apart from other factors that influence yield.

The development of satisfactory methods of comparing the yield of first-generation hybrids with that of their parents has been retarded by (1) a failure to fully appreciate the importance of individual diversity in hybrids, (2) the abnormal behavior of self-pollinated maize plants, and (3) the difficulty of securing for comparison hybrids and parents with identical ancestry. It is believed that the method here described avoids these difficulties and affords more accurate means of comparing first-generation maize hybrids with their parents.

The method is illustrated by an experiment in crossing two varieties of sweet corn in which it was found that the progeny from one hybrid ear yielded nearly double that of the other hybrid ear involved in the experiment. To have taken either ear alone would have led to entirely erroneous conclusions regarding the increase secured as a result of crossing. The increase in yield due to crossing as measured by the method here proposed was 31 per cent.

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NATURAL REVEGETATION OF RANGE LANDS BASED UPON GROWTH REQUIREMENTS AND LIFE HISTORY OF THE VEGETATION

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INTRODUCTION

Ideal range management would mean the utilization of the forage crop in a way to maintain the lands at their highest state of productiveness and at the same time afford the greatest possible returns to the stock industry. To maintain the maximum productivity, the annual herbage crop must be used in a manner which will not retard the growth or prevent the perpetuation of the most desirable forage species. On the other hand, if the stock industry is to receive the greatest possible returns at all times, the annual forage crop should be used when it is most needed and when the herbage is palatable and nutritious.

It is obvious from this that the requirements of the vegetation and the requirements of the stock are to a great extent antagonistic. Hence, unrestricted grazing, without regard for the vegetation or the locality, eventually results in decreased productivity, and often in denudation.

The decline in carrying capacity of our western grazing lands was brought about in part by injury due to trampling, but perhaps in greater part by premature grazing and overstocking. The growing herbage might be called a laboratory where plant nutrients are prepared, and the repeated removal of the foliage year after year during the fore part of the growing season means the destruction of this laboratory, which in turn means lack of nourishment for the vegetation, resulting in lowered vitality and an inability to produce seed.

The easiest way to overcome the deteriorating effect of premature grazing and overstocking, as well as of trampling, would be, of course, to eliminate grazing entirely. Obviously, however, such a procedure would be impracticable from the standpoint of the stock industry. Since this is so, the best means of solving the problem in a scientific manner is to

approach it as similar problems in farm practice are approached—that is, (1) by a careful study of the vegetation making up the forage crop, (2) by a study of the natural factors upon which depends the success or failure of the forage crop and its perpetuation, and (3) by a study to find a method of grazing which will both fully utilize the forage and at the same time protect it from deterioration.

Such studies were undertaken by the Forest Service in cooperation with the Bureau of Plant Industry during the spring of 1907 in the Wallowa Mountains of northeastern Oregon. While the intensive investigations were confined in the main to this one grazing region, the results have been applied elsewhere with success, notably in the Hayden National Forest in Wyoming. It is possible, of course, that the reproductive capacity of various forage plants may vary in different localities and also that there may be a difference in the behavior of plants on ranges grazed by sheep and those grazed by cattle and horses, any of which may affect the measure of success obtained by deferred grazing, but not the principles involved in the system.

The purely experimental studies were continued throughout the seasons of 1907, 1908, 1909, and 1910 and were followed by a practical application of the principles evolved to range management on lands within the Wallowa National Forest.

The system developed as a result of the studies—a combination of deferred and rotation grazing—is now being applied with minor variations to range lands throughout the National Forests, and promises to be of the greatest value in bringing about the efficient utilization of the forage resources.

This article gives in full the data upon which the new system is based. The area where the intensive studies were carried on is first described. Following this are given the life histories of the important forage species, including growth requirements and the factors influencing the establishment of reproduction. This in turn is followed by a discussion of the relative merits of different systems of grazing. Finally there is presented a rational and economical grazing system based upon the requirements of the forage plants and of the stock industry

TOPOGRAPHY AND SOIL

The Wallowa National Forest, within which the studies were carried on, is a region of high mountains, very irregular and broken. From the Grande Ronde and Wallowa Valleys, which bound the forest at about 3,000 feet elevation, the mountains rise to from 6,000 to 9,500 feet. On the upper reaches of the numerous domes, above the limits of forest growth, snow often remains throughout the summer. In this group of high, snowy peaks, within a radius of about 3 miles, rise nearly all of the streams from which the stock of the region are watered.

The forest exhibits three principal rock formations: Basaltic, granitic, and limestone. These give rise to as many different soil types, which in turn very largely determine the character and density of the vegetation. The best and most luxuriant vegetation is found upon the basaltic soils, which cover the greater part of the region, the comparatively recent lava flows from which they originate having buried the original formationsi n some places to a depth of several hundred feet. They are porous and very friable, admit of about average percolation, and retain water well. The granite and limestone soils, on the other hand, are poorly decomposed and lose moisture rapidly through percolation and evaporation. In consequence the vegetation is usually sparse, only the more drought-resistant plants being able to establish themselves. The limestones, mixed with shales, are the oldest and most restricted of the three formations. The granites, which are of a later period, form the peaks, crests, and soils of the very highest mountains.

CHARACTER AND DISTRIBUTION OF THE VEGETATION

Within the great altitudinal range between the lower valleys and higher mountains of the region wide differences naturally exist in the physical conditions which govern plant growth, and therefore in the character and composition of the growth itself. On the other hand, physical and climatic conditions, and consequently the vegetation, are strikingly similar within certain altitudinal limits, making it possible to divide the region into four climatic zones. Following Merriam's classification[1] these are:

Transition zone (yellow-pine association)	3,000 to 4,500 feet.
Canadian zone (lodgepole-pine association)	4,500 to 6,800 feet.
Hudsonian zone (whitebark-pine association)	6,500 to 8,500 feet.
Arctic-Alpine zone (Alpine-meadow association)	above 8,000 feet.

The altitudinal limits of these zones are not absolutely marked, since altitude does not wholly determine the character and composition of the vegetation. Hence, the limits given above should be considered only approximate for a given locality in this latitude.

The Transition Zone.—The Transition zone contains a number of coniferous tree species, the most characteristic being western yellow pine (*Pinus ponderosa*). Toward the upper limits of the zone yellow pine gives way to Douglas fir (*Pseudotsuga taxifolia*) and lowland fir (*Abies grandis*). As a rule, the timber is open, with considerable undergrowth of average palatability and nutritiousness, if grazed relatively early (Pl. XII).

Among the most characteristic and abundant herbaceous species is pine-grass (*Calamagrostis rubescens*). Other species which furnish a large

[1] Merriam, C. Hart. Life zones and crop zones of the United States. U. S. Dept. Agr., Div. Biol. Survey, Bul. 10, 79 p., 1 map. 1898.

part of the herbage on the lower, sparsely timbered lands are big bunch-grass (*Agropyron spicatum*), little bluegrass (*Poa sandbergii*), big bluegrass (*Poa scabrella*), and blue bunch-grass (*Festuca arizonica*). Germination and growth begin in the most exposed situations during the first week in April, and early in May the vegetation shows everywhere throughout the zone. Stock are usually not admitted before May 15.

CANADIAN ZONE.—The Canadian zone is characterized by lodgepole pine (*Pinus murrayana*), the predominant tree of the region. In many places the timber is so dense that there is little or no undergrowth of vegetation. Only the most tolerant shrubs and herbs can exist in the subdued light under the heavy timber, and such lands are of practically no value for grazing. In other places extensive areas of lodgepole pine have been burned over. Sometimes reproduction is established promptly, but where fire has consumed most of the organic matter in the soil, the reestablishment of vegetation of any kind is slow. Among the forerunners in the invasion of permanent species, fireweed (*Chamaenerion angustifolium*), a valuable sheep forage, and pearly everlasting (*Anaphalis margaritacea*), a plant of no forage value, are most common. Both growth and grazing begin in this zone fully 20 days later and end two weeks earlier than in the Transition zone below.

HUDSONIAN ZONE.—The Hudsonian zone, in contrast with the lands immediately below it, is open in character, the timber growing sparingly and in clumps. The predominating vegetation consists of grasses intermixed with various other palatable plants, as shown in Plate XIII, figures 2 and 3.

This zone probably covers a larger area than the two lower zones combined and supports most of the sheep permitted in the Wallowa Forest during the summer growing season. On account of the demands made upon this desirable range and because of the character of the forage, the Hudsonian zone has suffered more serious depletion than any other, and it was here that the most intensive study of revegetation was made.

The trees of the Hudzonian zone, most of which extend to the normal timber line, are Alpine fir (*Abies lasiocarpa*), whitebark pine (*Pinus albicaulis*), Engelmann spruce (*Picea engelmanni*), and mountain hemlock (*Tsuga mertensiana*). Whitebark pine is the most characteristic species, and its altitudinal distribution is so clearly marked that one can be certain wherever it is met that the conditions there are those of the Hudsonian zone. The timber grows in small, dense clumps, precluding an undergrowth of any but the most tolerant species.

Aside from the timber, vegetation is distinctly herbaceous and consists mainly of grasses and nongrasslike plants, commonly termed weeds. While a great many of the species are grazed to a limited extent at one time or another during the season, about 40 furnish 90 per cent of the

range forage. These, arranged in the order of their local forage value, are:

Mountain bunch-grass (*Festuca viridula*).
Little bluegrass (*Poa sandbergii*).
Short-awned brome-grass (*Bromus marginatus*).
Western porcupine, or needle grass (*Stipa occidentalis*).
Smooth wild rye (*Elymus glaucus*).
Tufted hair-grass (*Deschampsia caespitosa*).
Wild celery (*Ligusticum oreganum*).
Onion grass, or mountain bluegrass (*Melica bella*).
Red bunch-grass (*Agropyron flexuosum*).
Mountain wheat-grass (*Agropyron violaceum*).
Yarrow, or wild tansy (*Achillea lanulosa*).
Spiked trisetum (*Trisetum spicatum.*)
Butterweed (*Senecio triangularis*).
Coneflower (*Rudbeckia occidentalis*).
Wild buckwheat (*Polygonum phytolaccaefolium*).
Alpine timothy (*Phleum alpinum*).
Horsemint (*Agastache urticifolia*).
Wood rush (*Juncoides glabratum*).
Nuttall willow (*Salix nutallii*).
Fireweed (*Chamaenerion angustifolium*).
Mountain dandelion (*Agoseris glauca*).
Mountain onion (*Allium validum*).
Little needle grass (*Stipa minor*).
Wild onion (*Allium platyphyllum*).
Wild onion (*Allium collinum*).
Tall swamp-grass (*Carex exsiccata*).
False hellebore (*Veratrum viride*).
Valerian (*Valeriana sitchensis*).
Alpine redtop (*Agrostis rossae*).
Blue beardtongue (*Pentstemon procerus*).
Elk-grass (*Carex geyeri*).
Skunkweed, or Jacob's-ladder (*Polemonium humile*).
Sheep sedge (*Carex illota*).
Reed-grass (*Cinna latifolia*).
Woolly weed, or woolly hieracium (*Hieracium cynoglossoides*).
Onion grass, or mountain bluegrass (*Melica spectabilis*).
Wire sedge (*Carex hoodii*).
Tall meadow grass (*Panicularia nervata*).
Slender hair-grass (*Deschampsia elongata*).
Rush (*Juncus confusus*).
White foxtail (*Sitanion velutinum*).
Parry's-rush (*Juncus parryi*).
Rush (*Juncus mertensianus*).
Rush (*Juncus orthophyllus*).

Throughout the Hudsonian zone mountain bunch-grass (*Festuca viridula*) is by far the most abundant plant and the most desirable to revegetate. The relish with which several of the other species are grazed and their similar altitudinal range and abundance made it very difficult to determine which one ranks next in value, and the arrangement presented was not finally decided upon until after the third year's investigation.

Growth usually begins in the Hudsonian zone about the last week in June, and stock are given access to the lands early in July.

Arctic-Alpine Zone.—The Arctic-Alpine, or timberless, zone is not only unfavorable to tree growth, but to grazing plants as well. As shown in Plate XIV, figure 1, the zone is confined to the very highest crests and peaks, where the soil is shallow and poorly decomposed, the season of growth short, and nightly frosts common. Owing to the virtual lack of grazing in this region, it was not thought necessary to measure accurately the climatic factors.

The species of the Hudsonian zone are for the most part entirely absent in the Arctic-Alpine. Among the characteristic alpine plants are cat's-foot (*Eriogonum piperi*), whitlow-wort (*Draba aureola*), *Hoorebekia*

lyalli, hulsea (*Hulsea nana*), Alpine phacelia (*Phacelia alpina*), and false strawberry (*Sibbaldia procumbens*). Even these species occur sparingly.

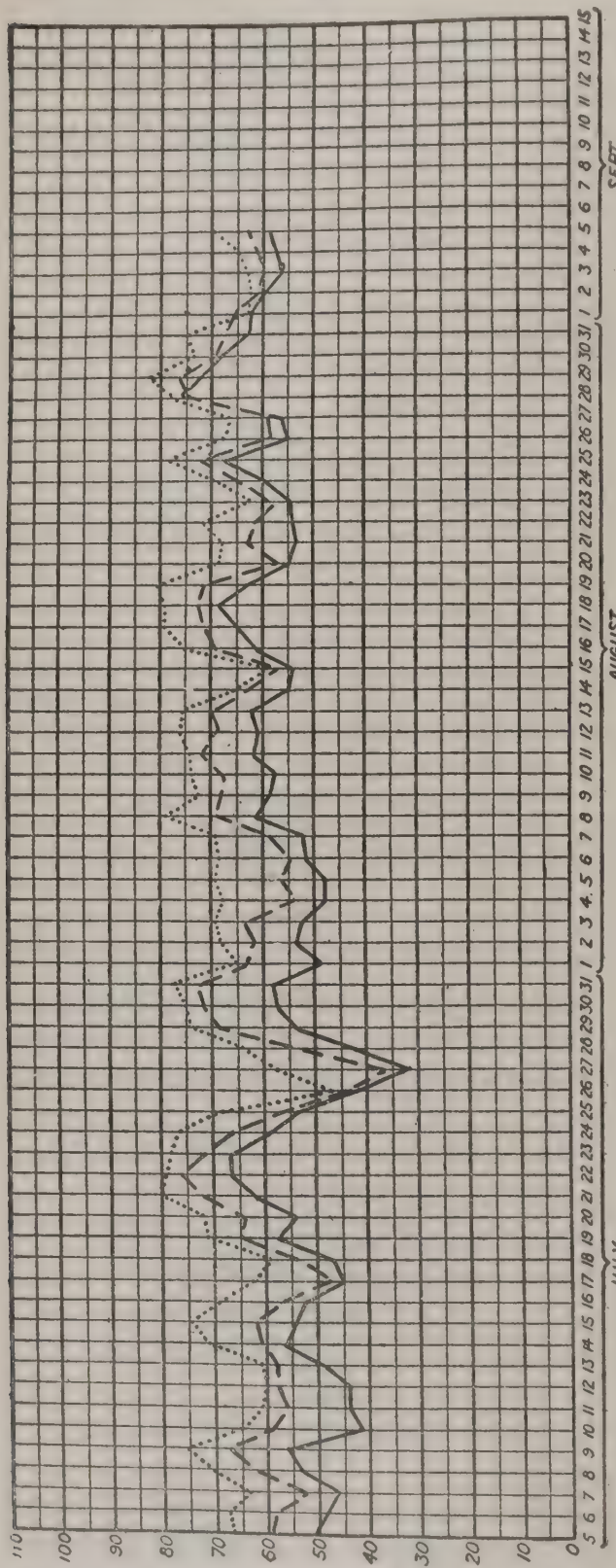

FIG. 1.—Curve showing the variation in the mean temperature in the Transition, Canadian, and Hudsonian grazing zones in 1909.

Growth does not usually begin until well into July and ceases about September 1. Naturally in this zone any species which succeeds in maturing viable seed must be vigorous and able to develop in a short time.

CLIMATE

Records of temperature, precipitation, and air humidity were kept in the Canadian, Transition, and Hudsonian zones during the main grazing season, in order to determine what differences in the season of growth they exhibit.

TEMPERATURE.—Figure 1 shows the mean temperatures of the respective zones derived from the daily maximum and minimum during the main growing season of 1909. While the mean temperatures in the three zones differed widely, there is a close relationship between the daily fluctuations. In all cases the mean is lower, usually by several degrees, in the Hudsonian zone than in the two lower ones, while the highest naturally comes in the Transition zone. The extremes from which the mean temperature was obtained show that

the variation in the maximum temperatures in the three zones is fully as great as in the case of the minimum. During the month of July, for example, the maximum temperature in the Hudsonian zone was 84°; in the Canadian, 90°; and in the Transition, 104° F. The highest temperature in the three zones during the entire growing season was 91°, 97°, and 105° F., respectively.

PRECIPITATION.—The higher temperatures characteristic of the less elevated lands are associated in the region of the study with a minimum precipitation. In the valley surrounding the Wallowa Mountains—that is, in the Transition zone—at an elevation of 3,600 feet, the annual precipitation is about 17 inches, the greater part coming in the spring, autumn, and winter. As a result, the vegetation often suffers for lack of moisture. In the Hudsonian zone, on the other hand, the larger amount of precipitation received is ample, and, with the exception of seedling plants, the vegetation is not affected by drought.

Figure 2 shows that the Transition and Canadian zones received 51.6 and 26 per cent less rainfall, respectively, than the Hudsonian. While

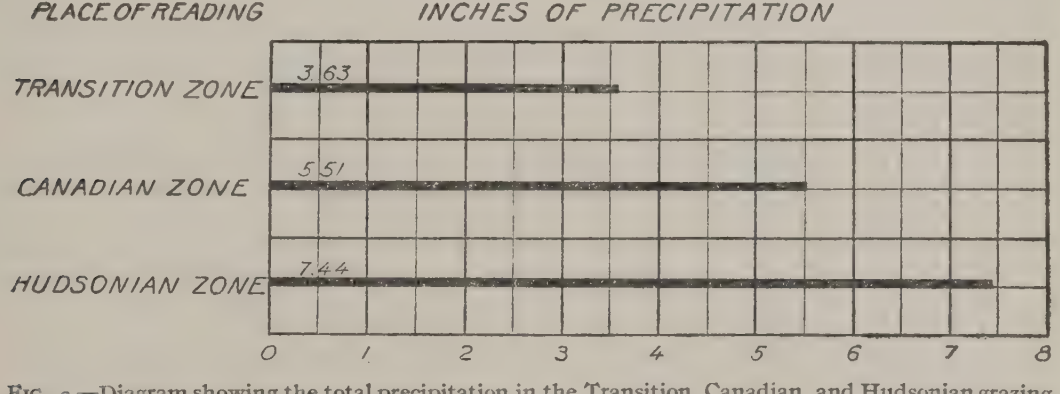

FIG. 2.—Diagram showing the total precipitation in the Transition, Canadian, and Hudsonian grazing zones during July, August, and September, 1909, inclusive.

the actual amount of precipitation during the growing season of 1909 was somewhat above normal, the ratio between the amount which fell in the different zones is similar to that of an average year. The greater amount of precipitation at the higher altitudes, together with the comparatively late date at which growth begins, accounts for the continuous development of the forage.

COMPARATIVE AIR HUMIDITY.—Since the Hudsonian zone has a relatively lower air temperature, a greater amount of precipitation, and a more humid soil than the lower zones, it would be natural to expect that the relative air humidity would be lower and transpiration less severe than in the lower areas. Figure 3, which gives the daily variations in air humidity derived from evaporation readings, shows this to be the case.

It was unexpectedly found that in the Hudsonian zone the evaporation was greater than in the Canadian zone immediately below. While the temperature and even the relative air humidity, as computed from psychrometer readings, were lower in the Hudsonian zone, the dense timber of the Canadian zone so interrupted the air currents as to materi-

ally diminish the evaporation there. In comparing the three zones, however, the evaporation in the Hudsonian and Canadian zones was found to be, respectively, 13 and 35.6 per cent less than that in the Transition zone. Owing to the relative great evaporation in the latter region, light showers, especially those which fall early in the day and are succeeded by a clear sky, are soon evaporated and so are of little value to plant growth. During a period of drought, other things being equal, a plant could not live nearly as long in the lower as in the higher elevations, on account of the excessive transpiration in the former locality.

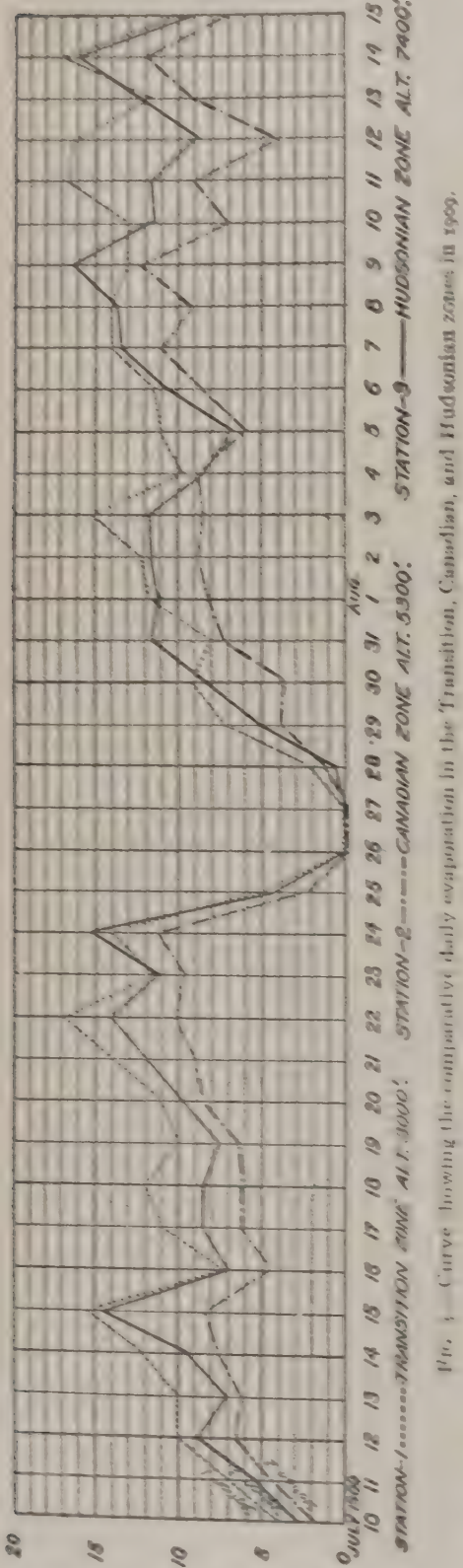

FIG. 1.—Curve showing the comparative daily evaporation in the Transition, Canadian, and Hudsonian zones in 1909.

To sum up the conditions peculiar to the Hudsonian zone as compared with the lower grazing type, the temperature is lower, the precipitation heavier, and the transpiration less. It is therefore easy to see that the relatively slow-growing and warmth-requiring vegetation in the lower lands could not thrive in the cooler and shorter growing season typical of the Hudsonian zone. Hence, only the most plastic and adaptable species of the Transition zone occur in the Hudsonian, and then only on the warmest and most exposed south slopes.

LIFE HISTORY OF THE FORAGE PLANTS

The growth requirements of range plants can best be determined by a study of individual species and the factors with which they have to contend from the time that they begin growth in the spring through the various stages of development to seed maturity, and then on to the permanent establishment and seed-bearing stage of the vegetation produced from seed of the original plants. The essential features of this almost double life cycle are: (1) Inception of growth, (2) flower-stalk

production, (3) development and maturity of seed, (4) viability of the seed crop, and (5) establishment of reproduction.

Owing to the relatively unfavorable conditions for plant growth and the demands made upon them for summer range, the high mountain lands are the ones most in need of revegetation. For this reason the life history of the forage plants and the factors affecting their activities were studied more intensively in the Hudsonian zone than elsewhere.

INCEPTION OF GROWTH

The time at which growth begins in the spring varies widely in the different zones. Thus, in the heart of the Hudsonian zone growth begins ordinarily about five weeks later than in the Canadian zone immediately below and seven weeks later than in the Transition zone. Even in the same zone the beginning of the growth period may vary by as much as 10 days from year to year, depending chiefly upon the climatic conditions during the spring, especially in May and June, but also to a certain extent upon the amount of snow accumulated during the winter. One year the early season may be characterized by warm, sunny days, and as a consequence the snow cover, especially on the more exposed situations, may disappear as early as June 20, though this is rather exceptional. In another year the snowfall in May and June may be as heavy as at any time during the winter. North and east exposures are always later in responding to growth than south and west aspects of the same elevation. Considerable difference exists also in the time when growth begins on different portions of the same slope.

The influence of local conditions on the inception of growth was well shown in the case of a north slope in the Hudsonian zone with an altitude at the crest of 7,850 feet and an incline of from 15° to 18°. With a succession of warm, sunny days the snow began to disappear first from the crest, then from the slope, and finally from the base. As the snow melted, growth began almost immediately. Before it had fairly begun on the slope, however, the crest showed a sparse covering of green, while a similar relation later existed between the slope and the base. In the early part of the season there was a marked difference between the amount of soil moisture at crest, slope, and base, the average percentages being 23.2, 27.6, and 39.9, respectively. As the season advanced, however, moisture conditions gradually became equalized, and in the latter part of the growing season the moisture content of the entire slope was practically uniform. For this reason, though growth began on the crest from three to six days earlier than upon the slope and from seven to nine days in advance of that on the base, toward the end of the growing period the vegetation as a whole presented a strikingly uniform appearance.

The period of growth resumption in each zone lasts ordinarily about 20 days. In the Hudsonian zone this period usually begins about June

25 and terminates about July 15. While climatic conditions have a direct influence upon the time when growth begins, the length of the growth-resumption period in a given locality is determined more by the vigor of the vegetation than by anything else. Where year after year the herbage has been removed prior to the time during which the nutrients necessary for spring growth are stored in the roots, growth begins several days later and vegetative development is strikingly less luxuriant than in the case of plants of the same species which have not been subjected to similar treatment.

FLOWER-STALK PRODUCTION

Under ideal conditions flower stalks begin to appear from 10 days to 2 weeks after growth has started. The stalks make a vigorous height growth, and there is a profusion of inflorescence, which is fertilized at an early date. Actually, however, the period of flower-stalk production is often retarded by cool temperatures and other climatic factors, so that the time the stalks begin to show may vary in different portions of the range as much as several days, similarly to the inception of growth. Obviously the two are closely related, an early and prolific herbage production being followed by an early and luxuriant flower-stalk development, and a late, scanty growth of herbage by a correspondingly late appearance of a few weak stalks.

The vigor of the vegetation and consequently the time and abundance of flower-stalk production are also strongly influenced by the way the lands are grazed. Close cropping, coupled with successive trampling prior to the full development of the plant, delays not only the flower-stalk production, but also the maturing of the seed crop in subsequent years. The period required for an overgrazed plant to regain its vigor depends on the amount of injury received and the situation in which it grows. Three seasons of protection from grazing are usually sufficient for herbaceous vegetation to recover its vigor fully.

To determine the actual difference in the time of flower-stalk production on closely grazed, moderately grazed, and protected areas, as well as the time required for overgrazed plants to recover their lost vigor, observations were made during three successive seasons of mountain bunch-grass areas grazed in different degrees, and on others completely protected from stock. The range selected was at an elevation of 7,300 feet in the heart of the Hudsonian zone. The unprotected or open range was grazed according to the usual practice, the forage crop being removed early in August each year, prior to maturity.

During the first year (1907) no difference was observed in the time of flower-stalk production on the protected and open ranges, since the vegetation on both had previously been weakened through grazing. In 1908 and 1909, however, there was a notable difference in flower-stalk production, as shown in Table I and Plate XV, figure 1.

TABLE I.—*Annual progression in the flower-stalk production on closed areas and open range*

Condition of range.	Period of flower-stalk production.			Estimated percentage of increase in flower stalks.		
	1907	1908	1909	1907	1908	1909
Closed to grazing....	July 10 to Aug. 20.	July 4 to Aug. 10.	July 1 to July 28.	0	20	60
Adjacent range open to grazing.	July 10 to Aug. 20.	July 8 to Aug. 20.	July 8 to Aug. 15.	.....	.....	.....

After the first year of protection mountain bunch-grass produced its flower stalks both earlier and more luxuriantly than on the adjacent range open to grazing. This was also true of other forage species. Though the advance in the time that the flower stalks appeared (4 and 7 days in 1908 and 1909, respectively) was not very great, the stalks were developed more uniformly, the total period required for the function on the protected area being 37 and 28 days in 1908 and 1909, respectively, and on the open area, 43 and 38 days.

To compare flower-stalk production on areas grazed in the usual way and on others protected from grazing until the seed crop can ripen, several small plots of mountain bunch-grass, 1 meter square, were clipped with shears just above the ground for three successive seasons. On half of the quadrats the herbage was clipped once each month, or three times during the growing season, while on the remaining plots cutting was not done until after seed maturity—about September 1. During the fourth and fifth seasons the herbage was undisturbed.

The results showed that in the case of the plots clipped monthly the vegetative growth decreased in abundance each successive season. In the fourth year the undisturbed herbage was exceedingly weak, short, and sparse. No flower stalks were produced until the vegetation had been given one full season of rest, and then only a few late weak stalks were sent up. On the plots clipped after seed maturity, however, the flower stalks were produced fully as early, as uniformly, and as profusely as in the case of the plants which had remained unmolested during the 5-year period. Herbage production was also equal to that on the protected areas.

On the open range, grazed early in the growing season, the flower stalks were produced at irregular periods, a few appearing early in July, the majority coming in August. On yearlong protected areas and on those protected until the seed crop had ripened, the stalks appeared early, practically all being in evidence before August 10.

Early and abundant production of flower stalks is of the utmost importance in seed production. Ordinarily from three to five weeks are

required for the proper development and "filling" of the seed after the flower stalks are produced. Climatic conditions usually become rather severe in the latter part of August, however, and flowers fertilized after the first week in August have not sufficient time to develop and mature their seed. Plants with low vitality are likely to produce flower stalks so late that the seed has no time to mature. A few species which reproduce vegetatively seem to have an inherent tendency toward irregular and late flower-stalk production, and such delay should not be confused with that due to low plant vigor. Of these species, pine-grass and yarrow, or wild tansy, require the longest time to produce flower stalks. On the lowest border of the Transition zone these species actually send up their flower stalks as early as July 10, and on the higher areas continue until inclement weather—about September 15—stops their activities.

PERIOD OF SEED MATURITY

Under the most favorable conditions the time required for the development of seed is about 25 days. The period varies slightly with the length of the growing season and is longest at the lower elevations. It also varies among different species, and the time given for ideal conditions should be considered as approximate.

Naturally the same factors which promote or retard the growth-resumption period and flower-stalk production also determine the time of seed maturity, though the last named fluctuates least, since toward the end of the growing season physical conditions, especially soil moisture and air and soil temperature, tend to become uniform throughout the range.

The length of the seed-maturing period varies widely from year to year as the result of the presence or absence of killing autumn frosts. In 1907 practically no seed of the more valuable perennial herbaceous species were matured in the Hudsonian zone until August 20, while in 1908 the ripening period came at least five days earlier. From this it might appear that the seeds were matured more slowly in 1908 than in 1907. This was not the case, however, the apparent difference being explained by the fact that in 1907 weather conditions after the first week in September were so unfavorable that the seeds which had not matured by that time were destroyed. In 1908, on the other hand, heavy frosts and low temperatures did not appear until September 20, and practically all the seed matured.

In 1908 seeds of mountain bunch-grass and other important species began to ripen on August 15, and by August 25 fully two-thirds of the crop had matured. After September 5 practically no immature seed were to be found, even on the cool north slopes at the higher altitudinal limits of the species. The secondary grazing plants, almost without exception, had matured their entire seed crop by September 10.

In 1909 the seed-maturity period began earlier than in the two preceding seasons. In the case of mountain bunch-grass and a few other species this difference amounted to as much as 10 days in the identical situations observed in previous years. As with the production of flower stalks, the latest period of seed maturity occurred in 1907. The seed of vegetation on the cool and moister north slopes invariably matured later than that on other exposures and on level land at the same altitude, the difference amounting to a week or 10 days. Elevation is, of course, influential in determining the time of seed maturity. Each increase of a thousand feet, other conditions remaining the same, brings about an approximate delay of a week. The chief factor in determining the time of seed maturity, however, as well as the size of the seed crop, is the vigor of the vegetation. Where the herbage had been grazed for several successive seasons when green and the vitality of the vegetation thus lowered, no seed was produced, or else the period of maturity came so late as to be seriously interfered with by frosts and low temperature. In contrast to this, the seed-ripening period on yearlong protected lands and on those not grazed until after seed maturity was much earlier and more uniform, while the amount of seed produced was notably greater.

On the unprotected range there was little difference in the time of seed maturity from year to year. On both the yearlong protected range and that grazed after seed maturity, however, the period came earlier each successive season, in direct ratio with the increase in vigor of the vegetation.

The importance of keeping the vegetation thoroughly vigorous is further exemplified by the clipping experiments. Where the herbage had been removed monthly for three successive seasons, no seed was developed in the fourth year when the plots remained undisturbed. On the other hand, on the plots clipped annually after seed maturity the seed crop was fully as large and matured at the same date as on lands from which stock were excluded.

The experiments show, therefore, that if the forage crop is left undisturbed until the seed has ripened, at which time the plants will have ceased growing, it will produce as large and as early a seed crop the following season as will vegetation on range not grazed at all. Clearly these facts are of the greatest importance in devising a system by which the forage may be grazed without interfering with seed production.

VIABILITY OF THE SEED CROP

The germinative power of the seed of the leading range plants was determined, in order to ascertain what reproduction might be expected under favorable conditions. In Table II, which gives the results of the tests, the high-range and low-range plants are grouped separately.

TABLE II.—*Average fertility of the seed of range plants from 1907 to 1909, inclusive*[a]

PLANTS OF THE HIGH RANGE

Name of plant.		Seed fertility (germination).
Common.	Scientific.	
		Per cent.
Mountain bunch-grass	Festuca viridula	12.2
Little bluegrass	Poa sandbergii	7.0
Short-awned brome-grass	Bromus marginatus	47.6
Tufted hair-grass	Deschampsia caespitosa	26.4
Onion grass, or mountain bluegrass	Melica bella	4.0
Reed-grass	Cinna latifolia	86.8
Alpine redtop	Agrostis rossae	36.0
Smooth wild rye	Elymus glaucus	21.2
Alpine timothy	Phleum alpinum	69.5
Western porcupine, or needle grass	Stipa occidentalis	27.0
White foxtail	Sutanion velutinum	69.5
Little needle grass	Stipa minor	29.8
Elk-grass	Carex geyeri	21.3
Tall swamp-grass	Carex exsiccata	15.2
Sheep sedge	Carex illota	27.5
Wire sedge	Carex hoodi	.....
Wood rush	Juncoides glabratum	7.5
Mountain onion	Allium validum	37.0
False hellebore	Veratum viride	24.0
Skunkweed, or Jacob's-ladder	Polemonium humile	42.0
Wild celery	Ligusticum oreganum	6.5
Blue beardtongue	Pentstemon procerus	18.5
Wild buckwheat	Polygonum phytolaccaefolium	9.5
Horsemint	Agastache urticifolia	22.2
Mountain dandelion	Agoseris glauca	36.0
Woolly weed	Hieracium cynoglossoides	10.9
Yarrow, or wild tansy	Achillea lanulosa	27.8
Coneflower	Rudbeckia occidentalis	21.8
Average germination		28.3

PLANTS OF THE LOW RANGE

Big bunch-grass	Agropyron spicatum	24.3
Pine-grass	Calamagrostis rubescens	69.6
Marsh pine-grass, or bluejoint	Calamagrostis canadensis	71.5
Mountain June-grass	Koeleria cristata	15.0
Slender hair-grass	Deschampsia elongata	41.6
Soft cheat	Bromus hordeaceus	48.2
Tall meadow-grass	Panicularia nervata	85.0
Geranium	Geranium viscosissimum	29.5
Fireweed	Chamaenerion angustifolium	21.5
Average germination		45.1

[a] In the case of a few species listed seed tests were made during two seasons only.

It will be observed that the viability of the seed of most plants is low. In the case of the important mountain bunch-grass, for example, barely more than one-tenth of the seed germinates. In a few cases mountain

bunch-grass seed showed a fair viability, the maximum germination obtained being 25.2 per cent, but the average of a great number of tests made under various degrees of temperature gave the figure in the table. In general, seed of the less desirable species, such as white foxtail and reed-grass, show a higher percentage of germination than that of mountain bunch-grass, short-awned brome-grass, and others. The germinative power of the seed was generally lowest in 1907, owing to the low vitality of the vegetation due to previous early grazing. In subsequent seasons on areas protected entirely from stock or until the seed had matured there was a pronounced increase in the germinative power of the seed.

It will be seen from Table II that, in general, seed fertility decreases with elevation, the average germination of the plants on the higher ranges being only 28 per cent, as against 45.1 per cent for those on the lower elevations. Even with the best conditions of growth and plant vigor, the vegetation of the region must struggle to mature its seed during a short and none too favorable growing season. The effect of exposure and of low vigor of the vegetation (as indicated by the date of maturity) on the germinative power of the seed is shown in Table III.

TABLE III.—*Effect of exposure and date of maturity upon germination of mountain bunch-grass*

Series No.	Source of seed.	Altitude.	Date of maturity.	Germination.
		Feet.		*Per cent.*
1	South exposure	7,400	Aug. 20	14.0
2	West exposure	7,400	Aug. 22	9.5
3	North exposure	7,300	Sept. 1	11.5
4	East exposure	7,350	...do.....	11.0
5	South exposure	7,400	Aug. 31	7.0
6	West exposure	7,400	...do.....	4.5
7	North exposure	7,300	Sept. 12	1.5
8	East exposure	7,350	Sept. 14	0

The data in this table bring out two important facts: (1) There is no difference in the vitality of the seed of mountain bunch-grass ripening before September 1, provided the variation in the maturing period does not exceed about 10 days. (2) There is a pronounced difference in the viability of seed which reaches maturity by September 1, as compared with seeds ripened September 10 or later, the latter showing practically no germinative power.

The same relationship between the germinative power of early and late-maturing seed was observed in the course of field sowing in the natural habitats, though in all such cases the germinative power of both classes of seed was higher.

The essential conclusions regarding seed germination are:

1. Even under the most favorable conditions the viability of the seed of practically all the forage species is low, especially on the high mountain lands.

2. Late resumption of growth in the spring and low plant vigor, both of which can be traced directly to premature grazing, result in a decrease in the amount of seed produced and in the germinative power of the seed itself.

3. If viable seed is to be produced, the vegetation must not be habitually deprived of its leafy foliage during the critical growing and food-storing period.

SCATTERING AND PLANTING OF THE SEED

But little time elapses between seed maturity and dissemination. This fact is highly advantageous, in that grazing may begin almost immediately after the seed matures without danger of having the crop consumed.

The distance the seed is carried from the parent plant depends chiefly upon the species and the wind. Grasses and grass-like plants, such as sedges and rushes, drop their seed near the parent plant. Those of plants like fireweed (*Epilobium* spp.) and *Crepis* spp., which are provided with bristly capillary hairs and pappus, and those of false hellebore, which are winged, are carried relatively great distances by the wind. Fireweed and dandelion do not grow in as dense stands as the grasses, but, as a rule, are more widely distributed over the range. About 90 per cent of the forage species depend primarily upon wind and water for the distribution of their seed. The remaining 10 per cent, of which huckleberry is an example, depend very largely upon animals for dispersal.

To insure reproduction of the forage plants, the seed must in some way get itself planted. Though nearly all seed will germinate on the surface of the ground where there is abundant moisture, the resulting seedling plants in a locality where the soil dries out early in the season are unable to extend their limited root systems deeply enough to reach the moist lower strata and consequently die from drought.

The size and character of the seed play an important part in the natural reproduction of range plants. The seed of some of the most important species, such as mountain bunch-grass, short-awned brome-grass, and wild celery, are large and light, and even though dropped promptly upon maturity in the autumn, months before germination takes place, are usually found uncovered on the ground in the spring. On the other hand, the seeds of wild onion and some of the sedges and rushes are smaller and heavier and have less difficulty in working into the soil. Among the valuable grasses observed, only one had become planted through natural means. This one exception was western porcupine grass, which is becoming securely established on the range not only in localities where it is abundant, but often on the tightly packed soils of denuded trails,

on hillside terraces formed by the trailing of sheep, and, in fact, everywhere that its seed is developed. In favorable situations 1 square meter of surface showed as many as 700 seedlings of porcupine grass in the spring of the year. This unusual aggressiveness is not due, as might be expected, to exceptionally strong seed habits, but chiefly to the morphology of the scale, or lemma, which closely envelops the seed. The scale is very rigid, with an awn about 1½ inches long protruding from the apex. At maturity this awn is tightly twisted, as shown in Plate XV, figure 2, but when moistened it untwists vigorously, causing the bent, needle-like point at the lower end of the scale to bore into the ground, the stiff, backward-turning hairs holding it in the earth when once started. The repeated twisting and untwisting of the awn with variation in the moisture finally results in the complete burial of the seed prior to the germination period.

Thus, if the seed of the valuable forage species is not planted by artificial stirring of the soil, undesirable species, such as white foxtail, may become established at the expense of the valuable range plants.

GROWTH AND ESTABLISHMENT OF REPRODUCTION OF FORAGE PLANTS

The production of a seed crop of high viability does not necessarily mean any material increase in the forage stand. The seedling plants are often seriously injured or destroyed in the fore part of the grazing season by low temperature and lack of soil moisture. Certain plants are not subject to as serious injury as others, and so the ultimate stand may consist of a single species. The growth and vitality of reproduction will be discussed under three heads: (1) "Development and loss of forage seedlings during first year;" (2) "Loss of forage seedlings during dormant period following first year;" and (3) "Growth and loss of forage seedlings during second and subsequent seasons."

DEVELOPMENT AND LOSS OF FORAGE SEEDLINGS DURING FIRST YEAR

During the first season of growth in the Hudsonian zone, approximately 10 weeks long, the seedlings do not grow tall enough to produce forage, though the young plants are sometimes cropped to a limited extent in the autumn. The height attained by mountain bunch-grass, as well as the root development, is shown in Plate XV, figure 3. It will be seen that the depth of the root slightly exceeds the height of that portion of the plant above ground. This plant represents about the average development of a forage seedling on well-drained and drier situations during the initial year of growth. On the lower elevations, owing to the longer growing season, the seedling plants usually attain much greater development than the one shown.

Observations extending over five successive seasons show that in normal years the low temperatures characteristic of the Hudsonian zone are responsible for considerable loss of seedlings during the first year of

growth. The extent of this influence is shown in figure 4. It will be observed that freezing temperatures occurred on three nights in July, 1909—namely, July 12, 17, and 27—the temperatures recorded being 30°, 23°, and 29° F., respectively. In August freezing temperatures occurred on the 1st, 4th, 20th, and 26th, the lowest being on the night of the 4th, when the temperature registered 28° F. Only on the nights of freezing temperature in July, however, was serious harm done, and then only to the young seedling plants. The greatest injury occurred on the more moist, but not marshy, situations, where the surface soil heaved as a result of alternate freezing and thawing. This action of the soil exposed portions of the roots of the seedlings, leaving them at the mercy of the sun and wind. In a few exceptional cases 50 per cent of the seedling stand was thus destroyed. The freezing temperatures during August were not destructive, since then the root systems were better developed and there was less heaving of the soil, on account of the lower moisture content of the surface layer.

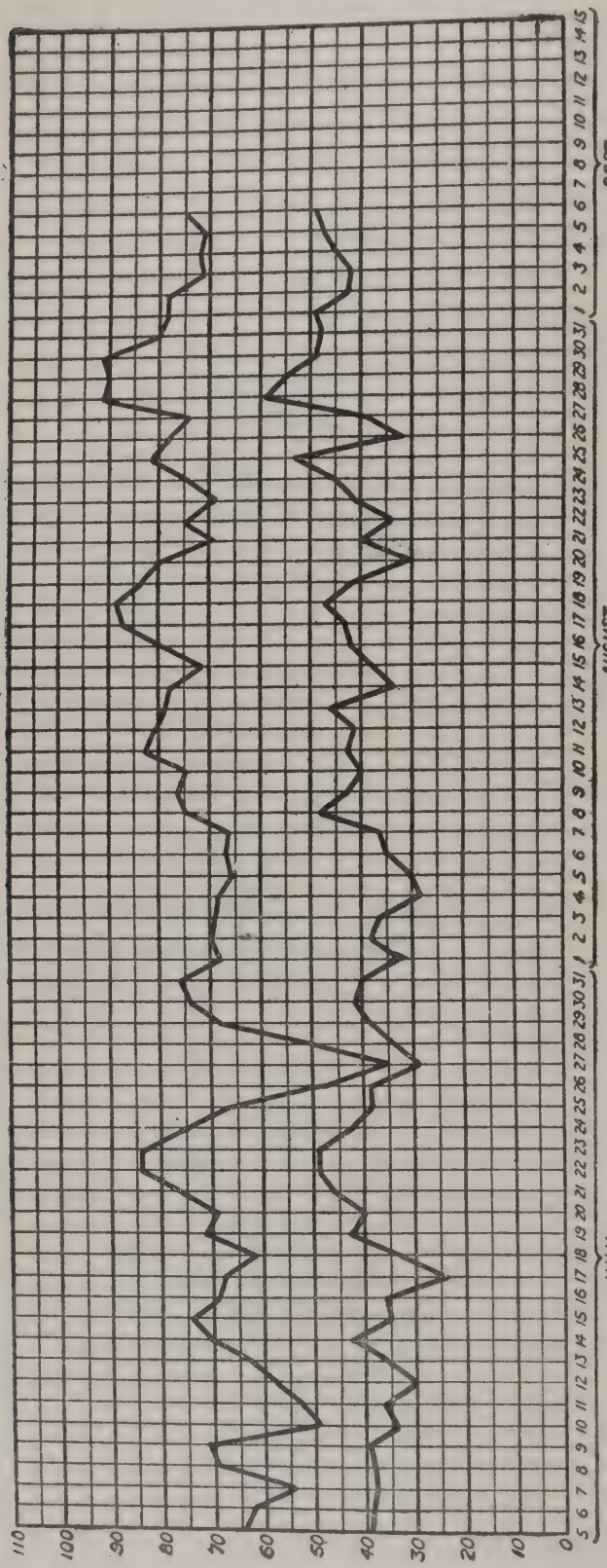

FIG. 4.—Curve showing the maximum and minimum temperature records in the Hudsonian zone (whitebark-pine association).

Because of the high elevation of the Hudsonian zone, the maximum temperature rarely exceeds 90° F. and, as a rule, does not seriously hamper the activities of the vegetation, though, in so far as it influences

the relative air humidity and increases transpiration and evaporation from the soil, it also decreases the available soil moisture.

With the limited amount of precipitation during the growing period in the region, the soil moisture gradually decreases as the season advances. Low temperatures and lack of moisture in the upper soil often work together to destroy seedlings, in that the seedling roots are first pushed up by the heaving of the soil during freezing to a point where there is not enough moisture available for growth.

The seedlings of no particular species seemed to suffer more seriously than any other in the same situation. Individuals which sprang from seed that had not found its way into the soil and which therefore had comparatively shallow root systems were, of course, the most seriously affected.

Plants of the same species growing in the same situation often exhibited contrasts in their ability to withstand drought, and when plants growing in different kinds of soil were observed, the contrast was great. This is shown in Table IV.

TABLE IV.—*Water content of soil at time of death of forage seedlings*

Name of plant, soil type, and situation.	Number of successive days of wilting.	Date of death.	Percentage of nonavailable water.	Average percentage of nonavailable water for each species.
Mountain bunch-grass:				
Basaltic clay loam—				
Quadrat station 4	8	July 22	6.8	.....
Seeded area	6	20	6.3	.....
Do	9	24	6.8	.....
Quadrat station 4, seedlings in flats	6	19	6.2	.....
Do	5	20	6.1	.....
Do	5	21	6.2	.....
Seeded area	10	23	7.2	6.51
Gravelly clay loam—				
Seeded area, seedlings in flats	4	19	5.4	.....
Do	5	19	5.7	.....
Do	3	20	5.4	.....
Do	6	.....	.....	.....
West slope	4	18	5.4	.....
Do	(?)	18	5.3	.....
South slope	5	19	5.4	.....
Do	4	18	5.6	.....
East slope	5	19	5.6	5.5
Western porcupine grass:				
Gravelly clay loam—				
West slope	6	21	5.2	.....
South slope	7	21	5.4	.....
East slope	6	21	5.2	5.26
Short-awned brome-grass:				
Gravelly clay loam—				
West slope	4	19	5.8	.....
South slope	3	19	5.6	.....
East slope	4	18	5.9	5.75

The figures in Table IV show two important facts: (1) In the finer soils a higher percentage of water is required to maintain the life of a seedling than in soils of coarser texture; and (2) mountain bunch-grass requires but little more available water than western porcupine grass, one of the deepest rooted species, to become established in a given soil. Mountain bunch-grass, moreover, will thrive on a soil containing less water than would be required for short-awned brome-grass. To judge from the nature of the situations invaded by mountain bunch-grass, the plant may safely be classed among the drought-resistant native species.

Numerous observations in 1909, supplemented by many counts on small unit areas in different range types and on different soils, showed that nearly the entire loss of seedlings occurred before August 1. After that date loss was prevented by cooler temperatures and by an increase in soil moisture resulting from precipitation. However, the loss before August 1 had been rather severe, varying from 20 to 70 per cent, according to the situation, with a general average of about 50 per cent.

In 1910 the seedling loss in the fore part of the season was approximately the same as in 1909, but the loss in the latter part was much greater, and only 25 per cent of the original stand remained vigorous and active in the autumn. This extensive loss was due to continued dry weather and high temperatures.

LOSS OF FORAGE SEEDLINGS DURING DORMANT PERIOD FOLLOWING FIRST YEAR

Table V shows that, in general, the loss of forage seedlings due to physical conditions from October 1, 1909, to July 1, 1910, approximately, was practically negligible. It will be seen that the heaviest seedling loss occurred on steep slopes, particularly on those where the soil was coarse and gravelly and the vegetation sparse. Quadrats 14 and 16 show this strikingly. The soil in quadrat 14, whose slope is 28.5° to the west, is very coarse and gravelly, and only one-tenth of the ground was covered with vegetation. In quadrat 16, with a slope of 3° to the south, the soil is mainly of clay loam, with a small amount of gravel, and three-tenths of the ground was covered with the same kind of vegetation as quadrat 14. The loss of seedlings on the two quadrats was 28.4 and 1.4 per cent, respectively. These losses were not due to severe temperatures, but primarily to heaving of the soil and erosion before growth began. Additional contrasts can be seen in quadrats 3 and 5, and 31 and 55.

TABLE V.—*Loss of forage seedlings during the dormant and winter period*

Number of each seedling species.	Quadrat No.	Slope and exposure.	Total number of vigorous seedlings remaining—		Loss.
			Autumn of 1909.	Spring of 1910.	
					Per cent.
80 Mountain bunch-grass	3	25° west	81	71	12.3
1 Western porcupine grass				.....	.....
244 Mountain bunch-grass	5	18° west	248	238	.....
4 Elk-grass				3	2.8
58 Mountain bunch-grass	13	16° west	59	55	7.0
1 Sickle sedge				0	.....
116 Mountain bunch-grass	14	28.5° west	116	83	28.4
61 Western porcupine grass	16	3° south	68	60	1.4
7 Yarrow				7	.....
116 Western porcupine grass	31	11° southeast.	138	92	26.3
17 Mountain bunch-grass				6	.....
30 Smooth wild rye	35	12° south	37	29	8.1
7 Western porcupine grass				5	.....
27 Smooth wild rye	38	4° southeast.	29	24	17.2
2 Little bluegrass				0	.....
138 Little bluegrass	42	11.5° south.	155	134	3.1
9 Smooth wild rye				8	.....
6 Yarrow				6	.....
2 Mountain bunch-grass				2	.....
128 Smooth wild rye	44	4° south	148	126	2.7
11 Yarrow				10	.....
9 Crepis (sp.?)				8	.....
91 Western porcupine grass	55	10° east	91	81	10.9

On steep hillsides where the original vegetation and network of roots had been seriously injured in the autumn by trampling, erosion carried the seedlings away or exposed portions of the more superficial (lateral) roots. Of the seedling loss during the resting period, 80 per cent was brought about in this way, though even this was nominal, averaging in the location studied only 7.3 per cent of the total stand.

Low temperatures were apparently responsible for the loss not directly due to gullying, but such loss was evident only in exposed situations. Practically all of the mountain lands are covered with a heavy blanket of snow before severe temperatures begin, which prevents excessive loss of water through the plant tissues aboveground and eliminates loss due to alternate freezing and thawing. No particular species appear to be especially immune to loss during the winter months. The roots of little bluegrass and sickle sedge seem to be exposed somewhat oftener than those of mountain bunch-grass, porcupine grass, short-awned brome-grass, and other species in the same situations. Mountain bunch-grass seedlings developed a rather unusually elaborate root system during the first year, which assisted in protecting them against adverse conditions.

GROWTH AND LOSS OF FORAGE SEEDLINGS DURING SECOND AND SUBSEQUENT SEASONS

During the second year nearly all species make vigorous growth, both above and below ground. Plate XVI shows the deep and spreading character of the roots of 2-year-old mountain bunch-grass (natural size) at the end of the second season. It will be seen that the roots are much longer than the leaf blades. The vertical roots reach well beyond the dry substratum during the most critical period of drought. The leaf blades, which number about 70 or 80 on the more vigorous individuals, are all basal, with an average length of about 4 inches, about half that of the deepest roots. This splendid root and herbage development provides the plant with abundant food-storage tissue, so that in the following season vigorous growth begins promptly.

During the third year the development of the plant is quite as marked as during the two preceding seasons (cf. Pls. XVI and XVII). Both roots and herbage grow rapidly from the first, though the growth of the former still greatly exceeds that of the leaf blades. Such development is essential, for the roots absorb moisture slowly, and where transpiration is great, nothing short of a well-developed root system can supply the plant with the moisture it requires. Owing to the depth and spread of the roots, the question of available soil moisture is not a serious one, since an ample supply exists 3 inches below the surface layer. At the start of the growing period there is a superabundant supply of moisture, and plants whose roots are well beneath the surface soil continue to extend them more deeply until the innumerable root hairs have worked themselves through the capillary spaces among the soil particles, thus insuring the plant against drought.

By the end of the third year mountain bunch-grass and other species complete their life cycles, and cease to be seedlings. Flower stalks and seed are then produced, as shown in Plate XVIII. At this time the plants are often as tall as older individuals. The specimen shown in Plate XVIII exhibits the maximum development attained, the average growth being shown in Plate XVII. The flower stalks (there are seldom more than three) of the 3-year-old plants as a rule are put forth a few days later than those of the parent or older plants. Consequently the seeds are not matured as early as are those of the longer established individuals, though the variation rarely exceeds five days. So extensive is the development of the plants by the second year of growth that the loss during that and subsequent seasons owing to climatic factors is negligible.

The facts derived from the study of the life history of the vegetation, which are important as a basis for a rational and practical grazing system, may be summarized as follows:

1. The flower stalks of the important grazing plants begin to appear about July 5 and are for the most part produced between that date and August 10. The more vigorous plants send up their flower stalks first. Plants weakened by annual close and early grazing do not produce flower stalks until late in the season, and then send up only a few.

2. The seeds begin to mature by August 15, and by September 1 the major part of the seed crop is ripened and disseminated. Plants weakened by close and early grazing do not mature seed unless the growing season is unusually long and exceptionally favorable.

3. The viability of the seed of most species is low. The germinative power varies with different species, but especially with the vigor of the plants. Those which make a weak vegetative growth produce seed of very low viability.

4. The seeds of the most valuable species lack means for working themselves into the ground, and, if reproduction is to be secured, they must be artificially covered.

5. In the Hudsonian zone the germination period begins about June 25, and growth begins generally by July 15.

6. During the first year of growth, a period of about 10 weeks, the forage seedlings make a vigorous development. Owing to the friability of the surface soil, however, and the superficial position of the roots at that time, there is rather a heavy loss of seedlings from freezing and drought during the spring period.

7. During the dormant periods there is virtually no loss of seedlings. The only factor causing loss is erosion.

8. In the second and subsequent seasons physical conditions are favorable to rapid development and growth of the young plants. By the end of the third season viable seed is produced.

DIFFERENT GRAZING SYSTEMS IN THEIR RELATION TO GROWTH REQUIREMENTS AND REVEGETATION

From the facts brought out by the life-history studies it is plain that a rational method of grazing should (1) avoid weakening the vegetation through continuous grazing prior to seed maturity; (2) utilize, so far as practicable, the trampling of the animals in planting the seed; and (3) provide for protecting the reproduction against heavy grazing until it is firmly established.

At the present time grazing on the National Forests is carried out under one of three more or less distinct systems: (1) Yearlong or season-long grazing year after year; (2) yearlong or season-long grazing combined with an occasional total restriction of stock during the entire year for the purpose of giving the forage plants a chance to reproduce; and (3) deferred grazing, which aims at a rotation in the time of using each portion of the range, each year allowing an area to reach seed maturity

before it is cropped, but grazing it after that period, in order to avoid loss of forage through nonuse and to assist reproduction by trampling in the seed.

In the following pages the comparative merits of the three grazing systems, from the standpoint of the requirements of the range plants for growth and reproduction, are discussed.

YEARLONG GRAZING

The term "grazing system" implies a definite plan of utilizing the forage crop in accordance with certain basic principles. Yearlong or season-long grazing, however, is characterized mainly by a lack of system, since it fails to provide for the removal of the herbage at any particular time in any locality. Its ultimate results to stock and the range are not considered.

It was this unrestricted grazing on National Forest lands prior to their inclusion that so seriously reduced the carrying capacity of the choice ranges. After the creation of the National Forests overstocking was eliminated as rapidly as the stockmen could meet the necessary reductions, and regular grazing seasons were established. Even under seasonal regulations, however, the prevailing practice of yearlong grazing has not been conducive to the most rapid improvement of the range. In northeastern Oregon sheep are permitted to enter the mountain grazing areas early in July, when mountain bunch-grass and most of the other palatable species begin to put forth their flower stalks. Up to August 1 the flower stalks are virtually as palatable as the leaf blades, and where the range is stocked to its full capacity, as it is in practically all cases, most of the stalks are removed prior to the formation of seed. Moreover, there is a tendency to graze the same lands prematurely each year, a practice which impairs herbage development. This not only prevents seed production, but also results in gradually decreasing the carrying capacity of the range through starvation of the forage plants. Prior to the time of seed maturity practically all of the range has been grazed over at least once, and, as a rule, only the vegetation on the inaccessible lands is allowed to mature seed.

After about August 1 the flower stalks are not eaten as a general rule, except in the case of certain moisture-loving species, such as butterweed (*Senecio triangularis*), but the vegetative portion, especially of the grasses, is so closely consumed as to prevent the manufacture of the food so essential to the development of the plants and the production of seed. Since the main seed-developing period in the Hudsonian zone comes in August, lack of an abundant food supply during the growth period is reflected in the low viability of the meager seed crop produced.

To determine which species are becoming established under the system of yearlong grazing, several typical areas, overgrazed in various degrees,

were studied in 1907 before the stock was turned on to them. Full notes were taken on about 300 plots, 1 meter square. The lands selected were of the open, parklike type, with a scattered growth of whitebark pine and occasional clumps of alpine fir. Mountain bunch-grass was the predominating herbage species. Certain portions of these ranges were seriously depleted, and the usual succession of early, aggressive annual weeds had replaced the original perennial type. At this altitude the annual plants are of little value for forage, though grazed to a limited extent in the spring when succulent and tender. On this account the annual plants are not included in Table VI, which gives the results of the two seasons' observations

TABLE VI.—*Seedling reproduction of perennial forage plants on mountain range areas in 1907 and 1908 under yearlong grazing*

1907

Date of observations.	Locality.	Slope and aspect.	Character of soil.	Percentage of moisture in soil.		Most characteristic species.	Percentage of ground covered by vegetation.	Number of unit areas counted.	Total number of seedlings.	Average number of seedlings per square meter.
				Minimum.	Average.					
August 5 and 6....	Stanley Range (elevation, 7,400 to 7,600 ft.).	2° west.....	Gravelly clay loam	11.4	20.1	Little needle grass, Alpine redtop, and sickle sedge.	10	45	428	9.51
August 6 and 7....	.....do.....	5° west.....	Light sandy loam.	10.6	20.1	Mountain bunch-grass, onion grass, or mountain bluegrass, and short-awned brome-grass.	50	36	183	5.08
August 9 and 10...	.....do.....	10° to 20° east.....	Clay loam.....	6.2	14.6	Elk-grass, sickle sedge, and short-awned brome-grass.	50	35	157	4.48
August 11.....	.....do.....	12° to 20° south...	.....do.....	7.1	13.6	Elk-grass and short-awned brome-grass.....	40	10	16	1.60
August 13.....	.....do.....	5° north.....	Sandy clay loam..	6.4	22.3	Mountain bunch-grass, Festuca ovina, and onion grass.	40	32	190	5.93
August 20.....	Sturgill Range (elevation, 7,700 to 7,800 ft.).	1° to 2° southwest.	Deep sandy loam .	23.4	30.2	Sickle sedge.....	(a)	25	674	26.96
August 20.....	.....do.....	5° south.....	Shallow, gravelly loam.	13.6	8.3	Mountain bunch-grass, little bluegrass, little needle grass, and Carex spp.	20	26	70	2.67
August 21.....	.....do.....	Level.....	Clay loam.....	7.1	8.2	Mountain bunch-grass, and big bunch-grass..	20	10	1	.10
August 21.....	.....do.....	5° to 10° south....	Gravelly clay loam.	10.3	6.5	Elk-grass and mountain bunch-grass.....	30	12	23	1.91
August 22.....	.....do.....	3° to 5° southwest.	.....do.....	9.6	8.9	Mountain bunch-grass, onion grass, and elk-grass.	30	22	55	2.50
August 23.....	.....do.....	10° to 15° south...	.....do.....	8.6	7.2	Elk-grass, and big bunch-grass.....	20	26	18	.69
August 23.....	.....do.....	5° to 10° east.....	Sandy, gravelly...	10.1	.....	Mountain wheat-grass (*Agropyron violaceum*) and elk-grass.	20	18	10	.55

July 20	Stanley Range (elevation, 7,400 to 7,600 ft.).	10° to 20° east	Clayey loam	10.8	17.3	Elk-grass, sickle sedge, and short-awned brome-grass.	60	16	69	4.3
July 20 and 21	do	1° west	Gravelly clay loam.	12.0	29.6	Alpine redtop, sickle sedge, and little needle grass.	12	22	286	13.0
July 22 and 23	do	5° west	Clay loam	5.9	24.0	Mountain bunch-grass, onion grass, and spiked trisetum.	20	26	130	5.0
July 25 and 27	do	15° to 20° east	do	8.6	19.3	Elk-grass, sickle sedge, and short-awned brome-grass.	50	14	74	5.3
July 27	do	5° southwest	do	9.2	26.4	Mountain bunch-grass, short-awned brome-grass, spiked trisetum, and onion grass.	15	24	170	7.1
July 28	Sturgill Range (elevation, 7,600 to 7,800 ft.).	1° to 2° southwest.	Deep clay loam	21.2	34.7	Sickle sedge, yarrow, little needle grass, and western porcupine grass.	(b)	17	401	23.6
July 29 and 30	do	3.6° southwest	Clay loam	9.0	22.0	Mountain bunch-grass, big bunch-grass, and little bluegrass.	40	12	50	4.2
July 31	do	5° south	Gravelly clay loam.	5.4	16.1	Little needle grass and big bunch-grass	20	14	49	3.5
August 3	do	Level	do	8.5	17.8	Little bluegrass and little needle grass	10	9	36	4.0
August 4 and 5	do	4° to 7° south	Clayey loam	9.3	11.8	Mountain bunch-grass, elk-grass, smooth wild rye, and horsemint.	25	11	36	3.3
August 8 and 9	do	5° east	Gravelly clay loam.	11.0	18.0	Mountain bunch-grass, onion grass, and little bluegrass.	40	12	76	6.4
August 10	do	Level	do	8.2	14.8	Big bunch-grass, western porcupine grass, and sickle sedge.	35	14	57.4	4.1
August 13	do	2° south	Black loam	16.4	28.2	Elk-grass, sickle sedge, slender hair-grass, and tufted hair-grass.	60	10	113	11.3

a Denuded.

b Almost denuded.

The data given in Table VI, supplemented by observations made in 1908 and 1909, show conclusively that on the typical lands studied the important perennial forage species are not being reestablished. For example, such important species as mountain bunch-grass, little blue-grass, and big bunch-grass gave a maximum count of 6.4 seedlings per square meter, as opposed to one of 26.96 for sickle sedge, a species of little value. Even on the lands which still support a fair stand of the original valuable forage virtually no reproduction is taking place. Mountain bunch-grass, which produces flower stalks at a relatively early date, and whose chances are therefore good for maturing a viable seed crop, shows no reproduction from seed where the ranges are grazed each year before the first week in August.

Practically all the seedlings on these ranges are of inferior species. Sickle sedge, an unpalatable but aggressive perennial, forms not less than nine-tenths of the total perennial seedling stand. This sedge matures a strong seed crop at a relatively early date and, in addition, perpetuates itself abundantly by offshoots from the rootstocks, which later develop seed. Besides sickle sedge, there was an occasional seedling of western porcupine grass, little needle grass, short-awned brome-grass, and slender hair-grass. The first three species are fairly good range plants, but the last named is grazed only to a limited extent early in the season. Probably because of this fact slender-hair-grass seedlings were more in evidence than any of the others.

The maximum seedling density occurred on old bed grounds, where the vegetative cover was exceedingly scarce. The average number of seedlings obtained per square meter for all counts made upon such lands was 26.96 and 23.6 in 1907 and 1908, respectively. This exceeds by about 50 per cent the seedling stand for any other type of range examined. There are three chief reasons why the seedling stand is dense on bed grounds: (1) The unpalatability of the parent species, coupled with early maturity of the seed; (2) thoroughness with which the seed is planted; and (3) relatively high water content of the soil.

The seed of sickle sedge usually matures and drops before August 1, and in consequence the plant is neither weakened nor the seed production interfered with by foraging animals. Though on most bed grounds the soil is hard-packed, on the particular ones examined it was loose and porous, and the trampling assisted in conserving its moisture by pulverizing the surface. During the main growing season in 1907 and 1908 the soil moisture content of the bed ground averaged 30.2 per cent, exceeding by 7.9 per cent that of any other locality studied, except the swales.

To sum up, it may be said that season-long grazing continued year after year seriously interferes with the growth of the vegetation, decreasing both the quantity and palatability of the forage crop. By the

failure of the forage plants to produce seed, reproduction is prevented, resulting in a gradual decline in the carrying capacity of the lands. Even under conservative use the carrying capacity of the range does not improve rapidly through reproduction of the more desirable species.

YEARLONG PROTECTION

To determine the practicability of reseeding the range through yearlong protection from grazing, five typical overgrazed areas, situated at various elevations from 3,000 to 7,500 feet, were selected for study. Each area was fenced in 1907, and observations were made during four successive seasons. The results from the areas in each zone are presented separately.

HUDSONIAN ZONE.—The areas closed to stock in the Hudsonian zone had been subjected to close yearlong grazing for several seasons (Pl. XXI, figs. 2 and 3), and because of the resultant low vitality of the vegetation practically no seed was produced during the first two seasons. In the third and subsequent seasons, however, a satisfactory seed crop of average viability was produced. The vegetative changes which took place in representative quadrats are shown in text figures 5 and 6.

It will be seen that at the time of their establishment the quadrats contained no perennial forage seedlings. The first year passed without any making their appearance. In 1909, however, 7 seedlings appeared after the germination period, but, as shown in figure 6, only 5 survived the subsequent dry season. On the denuded quadrat (fig. 6, quadrat 2) 2 mountain-bunch-grass seedlings came in during 1908, both of which succumbed later. In 1909, 10 seedlings were found in the spring, only 6 of which survived the season. Seed was produced in abundance each year, but for the most part remained on the surface of the soil. At the beginning of the study the quadrats were stocked with an inconspicuous and useless plant called knotweed (*Polygonum ramosissimum*), which is common on overgrazed ranges throughout the mountain-bunch-grass association. On the permanent quadrats this species no more than held its own, but on the denuded plots it increased prodigiously.

The contrast in the aggressiveness of reproduction of the annual and perennial species on protected areas, as shown in the case of mountain bunch-grass and knotweed, holds generally. The only perennial species which reproduced well under yearlong protection was western porcupine grass, the seed of which, as already pointed out, is planted by means of an awn attached to the floral glume.

The fact that practically no reproduction from seed was secured as a result of yearlong protection does not necessarily mean that such protection will not bring about an increase in the carrying capacity of the range. As a matter of fact the carrying capacity was increased through the production by the original perennial plants of more and longer leaf

blades and by an increase in the size of existing tufts or hummocks of tussock-forming plants. The leaf-blade increment is shown in Plate XXI, figures 2 and 3, where contiguous areas protected and grazed annually

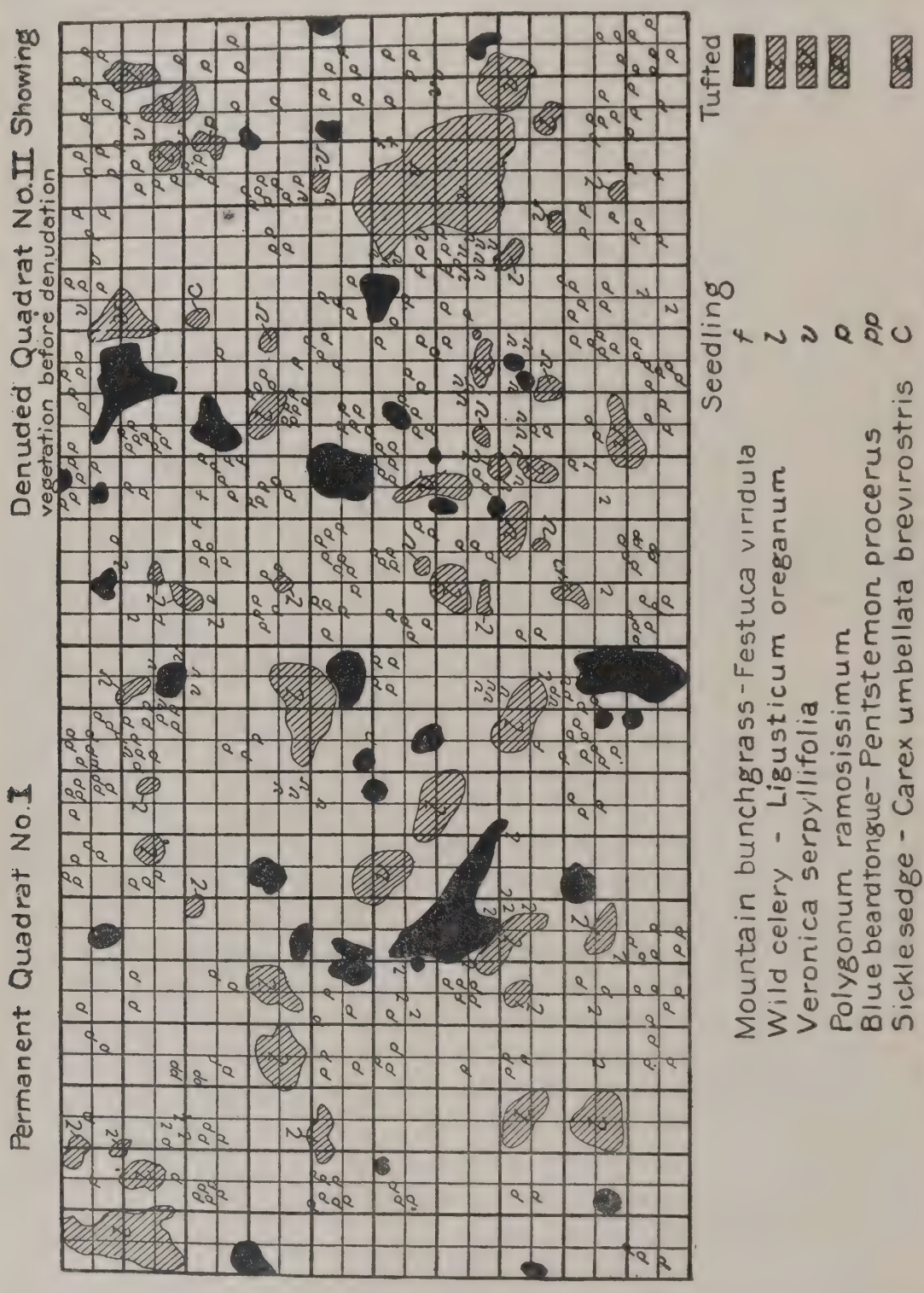
Permanent Quadrat No. I
Denuded Quadrat No. II Showing vegetation before denudation
Tufted
Seedling
Mountain bunchgrass - Festuca viridula f
Wild celery - Ligusticum oreganum l
Veronica serpyllifolia v
Polygonum ramosissimum p
Blue beardtongue - Pentstemon procerus pp
Sickle sedge - Carex umbellata brevirostris C

FIG. 5.—Chart of permanent and denuded quadrats 1 and 2 in station 4, established on July 3, 1907.

prior to seed maturity are compared. The leaf blades began to increase in number and length after the first year of protection and continued throughout the four years. The greatest development in the foliage came in the second year of protection, when the plant for the first time

was able to manufacture ample food. Many species of grass had doubled in length by the end of the fourth year.

The increase in actual stand or ground cover was due almost entirely to the enlargement of the tufts, and text figures 5 and 6 show that even under season-long protection the bunch-grasses and other valuable plants

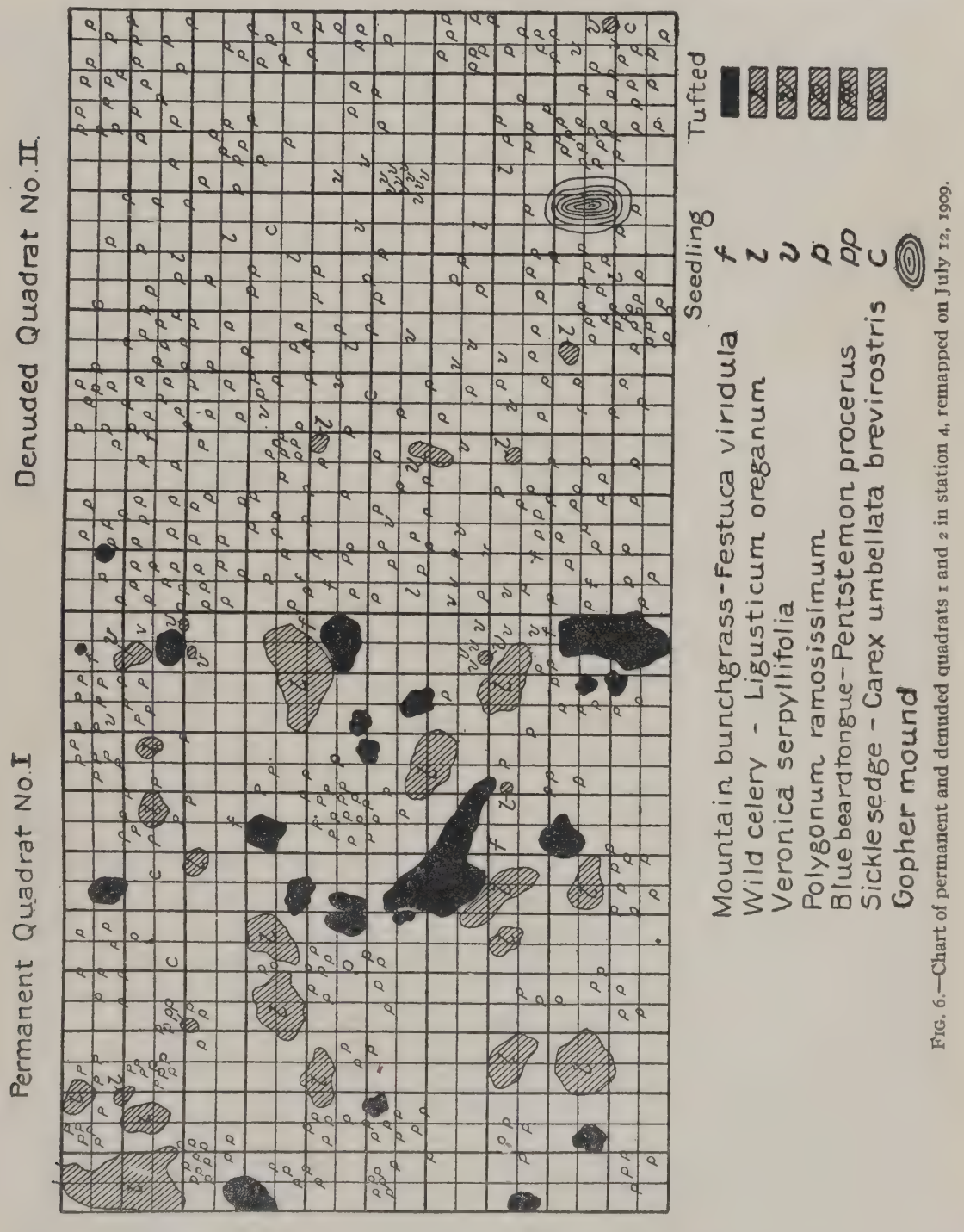

FIG. 6.—Chart of permanent and denuded quadrats 1 and 2 in station 4, remapped on July 12, 1909.

do not increase rapidly by this means. Planimeter measurements of the tufts showed an average increase in diameter of only 18 per cent for the third year of complete protection.

The young, small tufts were the only ones to increase noticeably in size. The tufts of mountain bunch-grass and most tussock-forming

species seldom measure more than 12 inches in diameter, and with the approach toward full development the annual increase becomes less and less. At just what age the hummock or tuft reaches full development is not definitely known, the age doubtless depending upon the species and situation.

TRANSITION ZONE.—Areas were carefully selected and fenced for study in the Transition zone in July, 1907. The range studied had been so seriously depleted that it was difficult to ascertain exactly what plant species constituted the main forage crop. The grasses found in more or less abundance at the time the plats were established were soft cheat, slender hair-grass, Olney's bluegrass, mountain June-grass, pine-grass, big bunch-grass, and western porcupine grass. Other important forage plants occurring sparingly were yarrow, alfileria (*Erodium cicutarium*), arnica (*Arnica cordifolia*), and geranium. Though the ground appeared to be nearly denuded, there was here and there a somewhat conspicuous stand of *Erigeron aureus*, stonecrop (*Sedum douglasii*), *Clarkia pulchella*, and Douglas knotweed (*Polygonum douglasii*).

During the first year of protection there was practically no plant invasion of the permanent quadrats (Pl. XX, fig. 3), but when the fall rains came—about September 1—the early stages of invasion became apparent on denuded areas. The plants to enter first were knotweed, an inconspicuous annual weed, *Clarkia pulchella*, *Erigeron aureus*, alfileria, soft cheat, western porcupine grass, and slender hair-grass, named in the order of their abundance. In the second year practically the same species entered the quadrats, with the addition of a mixed association of three perennial grasses—mountain June-grass, big bunch-grass, and pine-grass. Geranium, arnica, and stonecrop were also noted. At this time soft cheat, an annual species, was the most conspicuous and aggressive.

By the end of the third year, 1909, soft cheat had made such a rank growth that in certain portions of the protected area it had completely replaced the shorter annual weeds (cf. Pls. XX, fig. 3, and XXI, fig. 1). In the denuded quadrats certain perennial species, mountain June-grass, big bunch-grass, Olney's bluegrass, geranium, and yarrow, had also begun to appear as forerunners in the permanent establishment of perennial species. As a result of protection from grazing, the carrying capacity of the protected area as a whole had increased 150 per cent (Pl. XXII). This figure represents the increment in the ground cover within the quadrats rather than the weight of the forage produced. Fully four-fifths of the new growth was composed of annuals, with soft cheat easily predominating. Seeds of this species are small and, like those of western porcupine grass, work their way into the ground by means of special contrivances. The larger and lighter seeds of the perennial plants were found on the surface of the ground at the time of germination, and in consequence practically no seedlings of these species had been established. The perennial species had reproduced vegetatively, but the increase was by no means rapid.

On both the lower and more elevated lands the new forage resulting from yearlong protection consisted almost exclusively of annuals. A very small percentage indeed of the new stand was established through the reproduction of perennial plants. During the first two seasons of protection, even at the lower elevations, the perennials produced practically no fertile seed. In the third season viable seed were produced. The perennial vegetation, which previous to protection from grazing made such a weak growth that its presence was not observed, finally became conspicuous after one or two seasons of rest. Even under the most favorable conditions reproduction by the perennial species is slow, since the seeds are large and unable to work themselves beneath the surface of the soil. The nonuse of the forage under yearlong protection is a serious matter and would have to be considered before this system could be pronounced practicable. Moreover, the accumulation of inflammable material during the period of protection would result in increased fire danger. Under any circumstances it could not be carried out on a large scale without a radical readjustment of the stock industry.

To sum up the facts regarding yearlong protection: The system is not an efficient one, because the most valuable perennial species fail to reproduce by seed. While the carrying capacity of the land is increased, this increase is slow and does not compensate for the waste of the forage crop during the long period necessary for revegetation.

DEFERRED GRAZING

Unlike the two grazing systems just discussed, deferred grazing is based upon the requirements of the vegetation through practically a double life cycle, as defined in the section on the life history of the range plants.

To determine the effect of the deferred grazing system, the vegetation in the different grazing zones was studied for two successive seasons. Convenient areas which had been overgrazed in various degrees and which were large enough to support a band of sheep after the seed had ripened were closed to grazing from the beginning of the season until the seeds of the important species had matured. Upon maturity of the seed crop the range was grazed moderately by a band of sheep during the remainder of the season. The following year the same area was closed to sheep for the same period in order to give the seedlings an opportunity to develop a root system strong enough to withstand trampling and also to permit a second seed crop to be developed and disseminated in case the first year's seeding was unsuccessful. When the area had been satisfactorily reseeded, it was grazed early in the season, and a second area, large enough to maintain a band of sheep from the time of seed maturity to the end of the season, was reserved for deferred grazing. Results of a study to determine the abundance of seedling reproduction under the deferred grazing system, as compared with that under other systems, are shown in Table VII.

TABLE VII.—*Comparative seedling reproduction of perennial forage plants on similar areas under yearlong grazing and deferred grazing—Hudsonian zone*

Character of original vegetation.[a]	Percentage of ground covered by original vegetation.	Character of soil.	Slope and aspect.	Reproduction.					
				Yearlong grazing (1908).			Deferred grazing (1909).		
				Number of unit areas counted.	Total number of seedlings.	Average number of seedlings per square meter.	Number of unit areas counted.	Total number of seedlings.	Average number of seedlings per square meter.
Sickle sedge, yarrow, little needle grass, and western porcupine grass	(*b*)	Deep clay loam	1° to 2° southwest	17	491	24.6	9	261	29.0
Mountain bunch grass, big bunch-grass, and little bluegrass	40	Clay loam	3 6° southwest	12	50	4.2	7	220	31.4
Little needle grass and big bunch grass	20	Gravelly clay loam	5° south	14	49	3.5	10	393	39.3
Little bluegrass and little needle grass	10	do	Level	9	36	4.0	8	337	42.1
Mountain bunch grass, elk grass, smooth wild rye, and horsemint	25	Clayey loam	4° to 7° south	11	36	3.3	9	549	61.0
Mountain bunch grass, onion grass, and little bluegrass	40	Gravelly clay loam	5° east	12	76	6.4	8	486	48.1
Big bunch grass, western porcupine grass, and sickle sedge	35	do	Level	14	57	4.1	10	219	27.9
Elk grass, sickle sedge, slender hair-grass, and tufted hair-grass	60	Black loam	2° south	10	113	11.3	6	176	28.1
Average				12.4	101	7.5	8.4	330.6	39.6

[a] Named in the order of its abundance.

[b] Almost denuded.

SEEDLING REPRODUCTION SECURED

It will be seen that as a result of the deferred system of grazing the density of forage seedlings was from 2 to 10 times as great in 1909 as in 1908. Quite as important as the density was the identity of the seedling species. It will be recalled that where yearlong grazing was carried on, seedlings, aside from the annual weeds, consisted of two early-maturing annual plants, sickle sedge and slender hair-grass, together with a more valuable species, western porcupine grass. On the yearlong protected plots practically the only species were the few with small, heavy seeds, and the two porcupine grasses (*Stipa minor* and *S. occidentalis*) which are self-planting. Table VII shows that where deferred grazing was carried out the ground became stocked with seedling plants of all species which produce seed. The most valuable species, mountain bunch-grass, which failed completely under yearlong grazing and yearlong protection, responded exceptionally well. Its seedlings were found in all situations where there were parent plants to produce the necessary seed crop.

In the Canadian zone several of the valuable species which failed to reproduce under either of the other systems regenerated more or less abundantly under deferred grazing.

Although, under deferred grazing, forage seedlings were found wherever there were enough parent plants to produce the necessary seed, the proportion of the seedling stand which ultimately became established depended mainly upon the habitat and climatic conditions, as well as upon sufficient protection from grazing during the period of establishment.

LOSS OF SEEDLING REPRODUCTION BY GRAZING

To determine the extent to which moderate deferred grazing reduces the stand of valuable forage seedlings and whether the subsequent seedling stand resulting from the additional seed crop when thoroughly planted by trampling will offset the number of seedlings lost through grazing, observations were made on selected plots at medium and high elevations. The first observations recorded the character of the vegetation in and around each quadrat for a radius of 10 feet; the density of the herbaceous vegetation within and without the quadrat; the character of the soil and the slope and exposure; and the total number and identity of the seedlings within the quadrat, and their health vigor at the time of observation. With such data it was possible to account for any unusual loss resulting from subsequent grazing. It was recognized, for example, that a seedling, even when deeply rooted, is much more likely to be destroyed by trampling if it is situated on an abrupt hillside, especially in a denuded gravelly soil, than if situated in a level glade between tufts of grass with intertwining roots. Again, a seedling growing under adverse moisture conditions does not develop as elaborate and deep a root system as one which has received enough moisture to furnish the neces-

sary nutrients and so can not withstand as much disturbance of the soil. After the lands had been grazed and sufficient time had been allowed for the vegetation to recover, each quadrat was again observed and notes taken on the total number and identity of the forage seedlings which remained, the number of seedlings found dead, the number unaccounted for, the number whose recovery was doubtful, and the condition of the remaining seedlings at the time of the recounts.

At Medium Elevations.—The entire area studied slopes to the west and has a minimum altitude of 5,500 feet and a maximum which brings it into the lower Hudsonian zone. The topography is so irregular and there is so much down timber that the herbage can be grazed only under the most skillful open herding. The lower portion of the range is of the browse type, the much relished Nuttall willow predominating. The undergrowth consists of a host of weedy species, such as fireweed and its associates, with a scattering of smooth wild rye and short-awned brome-grass (Pl. XXIII). At the highest limits of Nuttall willow, seedlings of these species, on account of the shorter growing season, were not developed to the same extent as at the lower altitudes.

The forage stand in existence before and after grazing is shown in Tables VIII and IX.

TABLE VIII.—*Record of native forage seedling stand at medium elevation during the first week in August, preceding grazing*

Quadrat No.[a]	Character of vegetation in and around quadrat within radius of 10 feet.[b]	Density of vegetation.[c]		Character of soil.	Slope and exposure.	Total number of seedlings in quadrat.	Number of plants of each seedling species.	Condition of seedlings at time of observations.
		Within quadrat.	Without quadrat.					
1	Smooth wild rye and mountain bunch-grass.	1/10	2/10	Gravelly clay loam	16° west[d]	155	142 Smooth wild rye 13 mountain bunch-grass	Good, though a few had died previously.
2	Yarrow, smooth wild rye, valerian, and false hellebore.	2/10	3/10	Clayey loam	20° west	201	193 Smooth wild rye 7 Sickle sedge	Soil dry, seedlings indicating lack of water.
3	Smooth wild rye, skunkweed, valerian, and sickle sedge.	1/10	2/10	....do	24° west	43	1 Elk-grass 35 Smooth wild rye 1 Sickle sedge 1 Mountain bunch-grass	Many seedlings found dead, remaining ones showing lack of water.
4	Smooth wild rye, mountain bunch-grass, and valerian.	2/10	2/10	....do	18° west	125	105 Smooth wild rye 14 Mountain bunch-grass 6 Sedges	Fair condition.[e]
5	Mountain bunch-grass, smooth wild rye, and lupine.	2/10	5/10	Deep clay loam	18° west	61	52 Smooth wild rye 9 Mountain bunch-grass	Good.
6	Smooth wild rye, lupine, mountain June-grass, valerian, and sickle sedge.	4/10	4/10	....do	15° west	93	83 Smooth wild rye 6 Sickle sedge 4 Mountain bunch-grass	Do.
7	Short-awned brome-grass, smooth wild rye, and false hellebore.	5/10	5/10	Shallow, gravelly clay.	12° west	112	91 Short-awned brome-grass 19 Smooth wild rye 2 Sickle sedge	Do.
8	Short-awned brome-grass, mountain June-grass, and skunkweed.	4/10	4/10	Clay loam heavily impregnated with decaying wood.	12° west	80	64 Short-awned brome-grass 16 Smooth wild rye	Do.[e]
9	Smooth wild rye and valerian	1/10	4/10	Gravelly loam	20° southwest.	54	51 Smooth wild rye 1 Sickle sedge 1 Mountain bunch-grass 1 Elk-grass	Suffering from drought.
10	Smooth wild rye, elk-grass, and false hellebore.	1/10	1/10	....do	18° west	60	53 Smooth wild rye 6 Elk-grass 1 Sickle sedge	Good.
11	Smooth wild rye, valerian, and yarrow.	6/10	5/10	Clay loam	22° west	94	89 Smooth wild rye 5 Sickle sedge	Seedlings wilting, tips of leaf blades dead.
12	Smooth wild rye, false hellebore, and yarrow.	1/10	2/10	Slightly granitic clay loam.	20° west	66	58 Smooth wild rye 6 Sickle sedge 1 Mountain bunch-grass 1 Elk-grass	Do.

[a] These quadrats were remapped subsequent to grazing, the results of which appear in Table IX.
[b] Only the most predominant species are recorded under this heading. The species are arranged in the order of their abundance, the most predominant being given first.
[c] To represent the density of growth 10 is taken as complete ground cover.
[d] Situated on old sheep path.
[e] Gullying of the soil exposed the roots of a few seedlings.

TABLE VIII.—*Record of native forage seedling stand at medium elevation during the first week in August, preceding grazing*—Continued

Quadrat No.	Character of vegetation in and around quadrat within radius of 10 feet.	Density of vegetation.		Character of soil.	Slope and exposure.	Total number of seedlings in quadrat.	Number of plants of each seedling species.	Condition of seedlings at time of observations.
		Within quadrat.	Without quadrat.					
13....	Smooth wild rye and short-awned brome-grass.	2/10	4/10	Black clay loam.....	16° west.....	199	178 Smooth wild rye..... 19 Short-awned brome-grass..... 2 Sickle sedge.....	Fair, competition between rye-grass seedlings somewhat severe.
14....	Smooth wild rye, western porcupine grass, lupine, and valerian.	5/10	1/10	.....do.....	15° west.....	45	42 Smooth wild rye..... 3 Western porcupine grass.....	Good.
15....	Smooth wild rye and false hellebore...	5/10	3/10	Deep clay loam.....	13° west [a].....	105	101 Smooth wild rye..... 4 Short-awned brome-grass.....	Do.
	Average.....	.....	.....	.....	.....	100.8		

[a] Situated on old sheep path.

TABLE IX.—*Record of native forage seedling stand at medium elevation during the first week in September, after grazing*

Quadrat No.	Total number of seedlings remaining.	Total loss.	Percentage lost.	Number actually found dead.	Number not accounted for.	Number of plants of remaining seedling species.	Total number whose recovery is doubtful.[a]	Condition of remaining seedling stand at time of recount.	Remarks.
1	66	73	47.1	7	60	62 Smooth wild rye 4 Mountain bunch-grass	3	All native forage eaten closely in quadrat, leaving many roots exposed.	
2	98	103	51.2	11	90	92 Smooth wild rye 4 Sickle sedge 2 Elk grass	2	Sheep trail through part of quadrat, destroying and weakening many seedlings.	Sheep trail a foot wide and 4 inches deep.
3	12	31	72.1	3	27	10 Smooth wild rye 1 Sickle sedge 1 Mountain bunch-grass	1	Forage closely grazed and trampled...	All vegetation eaten into the ground.
4	54	71	56.8	8	60	44 Smooth wild rye 6 Mountain bunch-grass 4 Sedge	3	Trail through quadrat destroyed and weakened all seedlings in its path.	Many roots of seedlings exposed, due to soil washing.
5	28	33	54.1	5	27	26 Smooth wild rye 2 Mountain bunch-grass	1	Moderately grazed; seedlings in good condition.	
6	30	63	67.7	13	49	26 Smooth wild rye 4 Sickle sedge	1	Badly trampled; many seedling roots exposed.	
7	67	45	40.2	11	31	61 Short-awned brome-grass 6 Smooth wild rye	3	Closely grazed, but seedlings in good condition.	
8	74	7	8.6	4	0	68 Short-awned brome-grass 6 Smooth wild rye	3	Grazed very moderately; seedlings not injured.	Quadrat not in open, exposed position.
9	31	23	42.6	3	15	29 Smooth wild rye 1 Mountain bunch-grass 1 Elk grass	5	Grazed closely; seedlings in fair condition.	
10	31	29	48.3	3	22	28 Smooth wild rye 3 Elk grass	4	Moderately grazed; seedlings vigorous.	
11	46	48	51.0	7	39	44 Smooth wild rye 2 Sickle sedge	2	Very closely grazed and badly trampled; seedlings rather weak.	On open grazing land.
12	29	37	56.0	6	25	28 Smooth wild rye 1 Sickle sedge	6	Moderately grazed; seedlings vigorous.	
13	77	121	61.1	15	106	75 Smooth wild rye 2 Short-awned brome-grass	0	.....do.....	
14	22	23	51.1	3	12	19 Smooth wild rye 2 Short-awned brome-grass 1 Mountain bunch-grass	8	Badly trailed and trampled; seedlings fairly strong.	No seedlings left in trail.
15	47	58	55.2	12	40	46 Smooth wild rye 1 Short-awned brome-grass	6	.....do.....	Do.
Average.	48.5	50	50.9						

[a] Of the seedlings whose recovery from grazing or drought was doubtful, none were included in the number composing the "total number of seedlings remaining," as shown in column 2 of this table.

In Table VIII it will be seen that the seedling stand before grazing was rather dense, the average for all quadrats being 100.8 per square meter. Fortunately the less valuable species, such as sickle sedge, were the ones most weakened by drought, their condition, as will be noted, corresponding closely with the character of the soil, the more porous types supporting the less vigorous plants.

After grazing (Table IX) the average stand was reduced to 48.5 per square meter, a loss of 50.9 per cent. The heaviest loss was at the upper limit of the area, where the short growing season caused the seedlings to be less deeply rooted. At the lower elevations the heaviest loss was where the young plants were cropped. Many of the lateral roots were pulled out or broken, and death followed.

On account of the rather severe and uneven grazing, it was practically impossible to determine definitely what species were best able to withstand trampling. An examination of the root systems showed that smooth wild rye had almost invariably pushed its roots more deeply into the soil than any other species. Short-awned brome-grass also develops an unusually strong, deep, and spreading root, and showed ability to withstand trampling and to recover its vigor when portions of the root were pruned off below the surface, or even when segments of the rootlets were exposed to the air. Seedlings of mountain bunch-grass also withstood trampling comparatively well, notwithstanding the fact that at the time the range was cropped it was not so far advanced as the other species.

At High Elevation.—Owing to a great variety of conditions at the high elevations, 62 quadrats were established late in August before grazing. The range, which has a minimum altitude of approximately 7,500 feet, is distinctly herbaceous, the growth consisting primarily of grasses, with mountain bunch-grass and western porcupine grass predominating in the order named. (See text fig. 5.) In addition, there are several species of sedges and rushes, with a sprinkling of weeds and non-grasslike plants, especially in the moister situations.

At the time of the first observations nearly all the seedlings were in good condition, though dead individuals were often found in the drier situations. In other cases the terminal portions of some of the leaf blades were dead, but this did not necessarily indicate a weakened condition of the plant. The seedling stand, before and after grazing, is shown in Tables X and XI.

TABLE X.—*Record of native forage seedling stand at high elevations after August 15, preceding grazing*

Quadrat No.	Character of vegetation in and around quadrat within radius of 10 feet.[b]	Density of vegetation.[a] Within quadrat.	Density of vegetation.[a] Outside quadrat.	Character of soil.	Slope and exposure.	Total number of seedlings in quadrat.	Number of plants of each seedling species.	Condition of seedlings at time of observation.
1	Mountain bunch-grass and sickle sedge.	4/10	3/10	Coarse gravelly loam..	15° west.....	194	193 Mountain bunch-grass... 1 Sickle sedge.....	Good.
2	....do.....	2/10	1/10	Clay loam with some gravel.[c]	25° west.....	277	277 Mountain bunch-grass...	Some showed effects of drought.[c]
3	Mountain bunch-grass, sickle sedge, and western porcupine grass.	1/10	Denuded.	.....do.....	.....do.....	152	149 Mountain bunch-grass... 2 Western porcupine grass 1 Sickle sedge.....	Do.
4	.....do.....	5/10	...do.....	Clay loam.....	16° west.....	43	43 Mountain bunch-grass...	Good.
5	.....do.....	4/10	5/10	Gravelly clay loam....	18° west.....	572	560 Mountain bunch-grass... ... Elk-grass	Fair, but competition had restricted their growth.
6	Mountain bunch-grass, little bluegrass, and sickle sedge.	3/10	1/10	Deep loam rich in organic matter.	13° south.....	295	295 Mountain bunch-grass...	Fair; about 1/10 stand dead.
7	.....do.....	2/10	1/10	Clay loam.....	14° south.....	140	137 Mountain bunch-grass... 3 Sickle sedge.....	Good.
8	Mountain bunch-grass.....	3/10	5/10	Gravelly clay loam....	20° west.....	379	379 Mountain bunch-grass...	Portions of quadrat contained too dense growth for best development.
9	.....do.....	1/10	Denuded.	Very coarse gravelly loam.	.....do.....	193	193 Mountain bunch-grass...	Fair; some of terminal leaf blades dead.
10	Mountain bunch-grass, western porcupine grass, and sickle sedge.	5/10	...do.....	.....do.....	19° west.....	320	310 Mountain bunch-grass... 5 Western porcupine grass 5 Sickle sedge.....	Vigorous.
11	Mountain bunch-grass and wild buckwheat.	3/10	1/10	Clay loam.....	22° west.....	214	212 Mountain bunch-grass... 2 Sickle sedge.....	Fair; many seedlings had previously died.[d]
12	Mountain bunch-grass.....	3/10	2/10	Gravelly loam.....	18° west.....	152	149 Mountain bunch-grass... 3 Sickle sedge.....	Good.
13	Mountain bunch-grass and sickle sedge.	5/10	5/10	Clay loam.....	16° west.....	96	93 Mountain bunch-grass... 2 Sickle sedge..... 1 Sedge.....	Thrifty.
14	.....do.....	1/10	3/10	Gravelly clay loam....	28.5° west	172	171 Mountain bunch-grass... 1 Sickle sedge.....	Rather poor for lack of water.[e]

[a] To represent the density of growth, 10 is taken as complete ground cover.
[b] Only the most predominant species are recorded. They are arranged in their order of abundance.
[c] Soil lacking in organic matter.
[d] Soil had washed slightly.
[e] Gullying had been rather severe surrounding quadrat.

TABLE X.—*Record of native forage seedling stand at high elevation after August 15, preceding grazing*—Continued

Quadrat No.	Character of vegetation in and around quadrat within radius of 10 feet.	Density of vegetation.		Character of soil.	Slope and exposure.	Total number of seedlings in quadrat.	Number of plants of each seedling species.	Condition of seedlings at time of observation.
		Within quadrat.	Outside quadrat.					
15	Western porcupine grass, mountain bunch-grass, and sickle sedge.	Denuded.	2/10	Gravelly clay loam	16° west	90	85 Western porcupine grass 5 Sickle sedge	Vigorous.
16	Western porcupine grass, mountain bunch-grass, yarrow, and wild buckwheat.	3/10	5/10	Clay loam with little gravel.	3° south	130	121 Western porcupine grass 9 Yarrow	Do.
17	Mountain bunch-grass, western porcupine grass, and sickle sedge.	5/10	4/10	Clay loam	3° southwest	243	240 Western porcupine grass 2 Yarrow 1 Sickle sedge	Fair.
18	Western porcupine grass and mountain bunch-grass.	6/10	6/10	Clay loam	4° south	228	222 Western porcupine grass. 4 Sickle sedge 2 Mountain bunch-grass	Vigorous.
19	do	1/10	3/10	Fine clay loam	5° southwest	295	267 Western porcupine grass. 27 Mountain bunch-grass 1 Sickle sedge	Poor.
20	Western porcupine grass, yarrow, and mountain bunch-grass.	2/10	3/10	Very coarse, gravelly loam.	4° south	172	169 Western porcupine grass. 3 Mountain bunch-grass	Medium.
21	Western porcupine grass, mountain bunch-grass, and blue beardtongue.	1/10	2/10	Clay loam	5° southwest	52	52 Western porcupine grass.	Exceptionally vigorous.
22	Western porcupine grass, mountain bunch-grass, yarrow, and alum-root.	5/10	3/10	Coarse gravelly loam	3° south	320	312 Western porcupine grass. 6 Mountain bunch-grass 2 Sickle sedge	Competition too strong; many very weak.[a]
23	Western porcupine grass, mountain bunch-grass, and blue beardtongue.	5/10	1/10	Gravelly clay loam	4° south	169	166 Western porcupine grass. 2 Mountain bunch-grass 1 Sickle sedge	Good; above average.
24	Mountain bunch-grass, western porcupine grass, and yarrow.	5/10	1/10	do	1° south	218	195 Western porcupine grass. 21 Mountain bunch-grass 2 Sickle sedge	Good.
25	Western porcupine grass, mountain bunch-grass, and yarrow.	3/10	2/10	Very light gravelly loam.	4° south	145	129 Western porcupine grass. 13 Mountain bunch-grass 3 Elk-grass	Fair; some partly died back.
26	Mountain bunch-grass, western porcupine grass, and white foxtail.	1/10	1/10	Very coarse, gravelly clay loam.	4° east	52	27 Mountain bunch-grass 23 Western porcupine grass. 2 Sickle sedge	Some of mountain bunch-grass seedlings not vigorous.

27	Mountain bunch-grass, western porcupine grass, and blue beardtongue.	Denuded.	4/10	Light clay loam	3° southeast	103	81 Mountain bunch-grass. 17 Western porcupine grass. 5 Sedges.	Good.
28	Mountain bunch-grass and sickle sedge.	1/10	2/10	Scab and coarse gravelly loam.	4° east	32	24 Mountain bunch-grass. 4 Sickle sedge. 4 Western porcupine grass.	Do.
29	Mountain bunch-grass and western porcupine grass.	5/10	5/10	Clay loam	10° south	145	71 Western porcupine grass. 68 Mountain bunch-grass. 6 Sickle sedge.	Vigorous.
30	Western porcupine grass, mountain bunch-grass, and wild buckwheat.	5/10	2/10	Very coarse gravelly loam.	5° southeast	278	254 Western porcupine grass. 19 Mountain bunch-grass. 5 Short-awned brome-grass.	Medium.
31	Western porcupine grass and mountain bunch-grass.	Denuded.	3/10	Coarse gravelly clay loam.	11° southeast	486	417 Western porcupine grass. 68 Mountain bunch-grass. 1 Elk-grass.	Medium; seedlings too thick in places.[b]
32	Short-awned brome-grass, western porcupine grass, mountain bunch-grass, and wild buckwheat.	2/10	3/10	do	6° east	66	51 Short-awned brome-grass 6 Western porcupine grass. 5 Sickle sedge. 4 Mountain bunch-grass.	Excellent.
33	Mountain bunch-grass and western porcupine grass.	1/10	2/10	do	9° south	88	83 Western porcupine grass. 4 Mountain bunch-grass. 1 Sickle sedge.	Medium; some show severe effects from drought.
34	Mountain bunch-grass, smooth wild rye, and western porcupine grass.	1/10	6/10	Scabby, gravelly loam.	2° southeast	54	42 Smooth wild rye. 6 Western porcupine grass 3 Mountain bunch-grass. 3 Sickle sedge.	Rye-grass seedlings so tall as to furnish forage.
35	Smooth wild rye, western porcupine grass, and mountain bunch-grass.	1/10	5/10	Gravelly loam	12° south	73	46 Smooth wild rye. 25 Western porcupine grass. 2 Sickle sedge.	Good.
36	Mountain bunch-grass, western porcupine grass, and smooth wild rye.	2/10	4/10	Coarse, gravelly clay loam.	2° southeast	101	81 Smooth wild rye. 9 Western porcupine grass. 7 Mountain bunch-grass. 4 Sickle sedge.	Medium.
37	Western porcupine grass, mountain bunch-grass, smooth wild rye, and sickle sedge.	1/10	5/10	Rocky "scabland," with shallow loam.	10° south	246	220 Western porcupine grass. 14 Mountain bunch-grass. 10 Sickle sedge. 2 Smooth wild rye.	Vigorous.
38	Smooth wild rye and little bluegrass.	(c)	2/10	do	4° southeast	37	31 Smooth wild rye. 3 Little bluegrass. 3 Yarrow.	Vigorous; little bluegrass somewhat weak.
39	Western porcupine grass, mountain bunch-grass, and yarrow.	3/10	2/10	Very coarse gravelly loam.	14° south	217	213 Western porcupine grass. 3 Mountain bunch-grass. 1 Yarrow.	Good; have made exceptional growth.
40	Little bluegrass, big bunch-grass, and slender hair-grass.	1/10	4/10	Fine, gravelly clay loam.[d]	Level	135	120 Little bluegrass. 12 Slender hair-grass. 1 Smooth wild rye. 1 Yarrow. 1 Big bunch-grass.	Drought has weakened many.

[a] Soil had washed slightly; seedlings too thick.
[b] Root competition severe in some parts of quadrat.
[c] Nothing but seedlings.
[d] Soil compact and somewhat baked.

TABLE X.—*Record of native forage seedling stand at high elevation after August 15, preceding grazing*—Continued

Quadrat No.	Character of vegetation in and around quadrat within radius of 10 feet.	Density of vegetation.		Character of soil.	Slope and exposure.	Total number of seedlings in quadrat.	Number of plants of each seedling species.	Condition of seedlings at time of observation.
		Within quadrat.	Outside quadrat.					
41	Western porcupine grass, small bluegrass, mountain bunch-grass, and blue beardtongue.	Denuded.		Clay loam	12.5° south	78	62 Porcupine grass. 7 Little bluegrass. 5 Blue foxglove. 3 Smooth wild rye. 1 Yarrow.	Good.
42	Little bluegrass, short-awned brome-grass, and mountain bunch-grass.	1/10	3/10	Clay loam	11.5° south	185	148 Little bluegrass. 13 Yarrow. 11 Short-awned brome-grass. 10 Smooth wild rye. 3 Mountain bunch-grass.	Little bluegrass; poor for lack of water.
43	Mountain bunch-grass, western porcupine grass, and sickle sedge.	2/10	4/10	Gravelly clay loam	14° south	94	81 Western porcupine grass. 5 Little bluegrass. 5 Sickle sedge. 3 Mountain bunch-grass.	Medium.
44	Smooth wild rye, yarrow, and mountain dandelion.	5/10	2/10	Clay loam	4° south	204	180 Smooth wild rye. 13 Mountain dandelion. 11 Yarrow.	Do.
45	Western porcupine grass, mountain bunch-grass, and blue beardtongue.	Denuded.	5/10	Scabby soil, shallow loam.	9° west	321	321 Western porcupine grass.	Rather badly wilted.
46	Mountain bunch-grass, little bluegrass, yarrow, and blue beardtongue.	1/10	4/10	Clay loam[a]	3° south	74	57 Mountain bunch-grass. 13 Mountain dandelion. 3 Yarrow. 1 Blue beardtongue.	Fair; some roots gullied out of ground.
47	Western porcupine grass, smooth wild rye, and mountain bunch-grass.	1/10	4/10	Gravelly clay loam	11° south	87	61 Western porcupine grass. 23 Alpine redtop. 3 Smooth wild rye.	Very good.
48	Mountain bunch-grass, western porcupine grass, and big bunch-grass.	1/10	2/10	Very coarse, gravelly clay loam.	3° south	36	28 Mountain bunch-grass. 5 Yarrow. 2 Big bunch-grass. 1 Smooth wild rye.	Do.
49	Western porcupine grass, mountain bunch-grass, smooth wild rye, and yarrow.	1/10	6/10	Gravelly clay loam	9° south	287	274 Western porcupine grass. 9 Yarrow. 4 Mountain bunch-grass.	Good.

50	Smooth wild rye, mountain bunch-grass, and yarrow.	1/10	6/10	....do....	1° south....	67	49 Smooth wild rye.... 16 Yarrow.... 2 Mountain bunch-grass...	Do.
51	Western porcupine grass, mountain bunch-grass, and yarrow.	1/10	1/10	Rocky gravelly loam..	13° south....	166	142 Western porcupine grass. 18 Mountain bunch-grass... 6 Sickle sedge....	Medium. Many had previously died.
52	Mountain bunch-grass and a number of annual weeds.	1/10	3/10	....do....	8° south....	24	24 Mountain bunch-grass...	Generally good.
53	Mountain bunch-grass and yarrow.	2. 5/10	2/10	Scabby clay loam....	13° east....	39	34 Mountain bunch-grass... 5 Sickle sedge.	Medium.
54	Mountain bunch-grass and western porcupine grass.	1/10	3/10	....do....	4° east....	36	30 Mountain bunch-grass... 5 Western porcupine grass. 1 Sedge....	Excellent.
55	Western porcupine grass and annual weeds.	4/10	2/10	Rocky clay loam [b]....	10° east....	321	321 Western porcupine grass.	Very poor, 50 per cent having previously died.
56	Mountain bunch-grass, western porcupine grass, and short-awned brome-grass.	2/10	3/10	Rocky, gravelly clay loam.	3° east....	33	28 Mountain bunch-grass... 3 Western porcupine grass. 2 Short-awned brome-grass	Good.
57	Mountain bunch-grass....	5/10	5/10	Clay loam....	9° east....	32	32 Mountain bunch-grass...	Vigorous.
58	Mountain bunch-grass and western porcupine grass.	2/10	1/10	Rocky clay loam....	5° east....	45	40 Mountain bunch-grass... 5 Western porcupine grass.	Good.
59	Mountain bunch-grass and western porcupine grass.	2. 5/10	2/10	Clay loam....	22° east....	27	17 Mountain bunch-grass... 9 Western porcupine grass. 1 Sickle sedge....	Medium.
60	Mountain bunch-grass....	2/10	1/10	Deep clay loam [c]....	6° east....	30	29 Mountain bunch-grass... 1 Western porcupine grass.	Vigorous; unusually large.
61	....do....	1/10	1/10	....do....	7° east....	29	29 Mountain bunch-grass...	Vigorous.
62	Mountain bunch-grass and western porcupine grass.	1/10	2/10	Gravelly clay....	8° east....	35	34 Mountain bunch-grass... 1 Western porcupine grass.	Good.
	Average seedling stand....					155.3		

[a] Surface soil removed by washing to depth of 2 inches.
[b] Soil very dry; terminal part of many leaf blades dead.
[c] Soil very compact and slightly baked.

TABLE XI.—*Record of native forage seedling stand at high elevations after September 1, after grazing*

Quadrat No.	Total number of seedlings remaining.	Total loss.	Percentage lost.	Number found dead.	Number not accounted for.	Number of plants of remaining seedling species.	Number whose recovery seemed doubtful.[a]	Condition of remaining seedling stand at time of recount.	Remarks.
1	119	75	38.6	6	69	119 Mountain bunch-grass	3	Much weakened through close grazing and trailing.	
2	162	115	41.5	10	105	162 Mountain bunch-grass	6	do	
3	81	71	46.7	3	68	80 Mountain bunch-grass 1 Western porcupine grass		Fair; showed lack of moisture.	
4	26	17	39.5	2	15	26 Mountain bunch-grass	1	Medium strong	
5	248	324	56.6	15	309	244 Mountain bunch-grass 4 Elk-grass	21	Medium; closely grazed	Drought had previously impaired the seedling growth.
6	122	173	58.6	5	168	122 Mountain bunch-grass	33	Vigorous	
7	54	86	61.4	7	89	53 Mountain bunch-grass 1 Sickle sedge	5	Generally strong, some much weakened.	
8	183	196	51.7	23	173	183 Mountain bunch-grass	11	Fair	Severely grazed.
9	96	87	47.5	2	85	96 Mountain bunch-grass	8	Fair; seedlings previously weakened for lack of water.	
10	147	173	54.0	6	167	145 Mountain bunch-grass 1 Western porcupine grass 1 Sickle sedge	7	Fair	
11	94	120	56.0	10	110	193 Mountain bunch-grass 1 Sickle sedge	4	Terminal part of some leaf blades dead.	Washing of soil had exposed some of the seedling roots.
12	89	63	41.4	4	59	89 Mountain bunch-grass	5	Thrifty	
13	59	37	38.5	2	35	58 Mountain bunch-grass 1 Sickle sedge	2	Seedlings much weakened for lack of water.	
14	116	56	32.5	4	52	116 Mountain bunch-grass	4	Vigorous	
15	45	45	50.0	.....	45	44 Western porcupine grass 1 Sickle sedge	4	do	
16	68	62	47.7	12	50	61 Western porcupine grass 7 Yarrow	6	Medium strong	Weakness of seedlings due, in part, to poor moisture conditions.
17	107	136	55.9	3	133	105 Western porcupine grass 2 Yarrow		Vigorous	
18	145	83	36.4	11	72	140 Western porcupine grass 2 Mountain bunch-grass 3 Sickle sedge	5	do	
19	123	172	58.3	2	170	122 Western porcupine grass 1 Sickle sedge		Most of seedlings weak	
20	96	76	44.2	13	63	95 Western porcupine grass 1 Mountain bunch-grass	7	Fair; poor soil and drought the main cause.	
21	30	22	42.3	8	14	30 Western porcupine grass	4	Vigorous	

22	204	116	36.2	4	112	198 Western porcupine grass 6 Mountain bunch-grass	2	Weakened badly by trampling.	Many roots exposed because of trampling and close seedling stand.
23	101	68	40.2	9	59	100 Western porcupine grass 1 Sickle sedge	1	Medium vigorous	
24	117	101	46.3	3	98	111 Western porcupine grass 6 Mountain bunch-grass	3	Good	
25	69	76	52.4	2	74	60 Western porcupine grass 7 Mountain bunch-grass 2 Sickle sedge	7	Fair; closely grazed	
26	17	35	67.3	3	32	9 Western porcupine grass 8 Mountain bunch-grass	3	Poor	Sheep had trailed through quadrat, destroying many seedlings.
27	30	73	70.8	6	67	29 Mountain bunch-grass 1 Western porcupine grass	2	Good	
28	14	18	56.2	4	14	8 Mountain bunch-grass 5 Western pocupine grass 1 Sickle sedge	4	Much weakened	
29	72	73	50.3	2	71	50 Western porcupine grass 20 Mountain bunch-grass 2 Sickle sedge	1	Severely trampled and weakened.	
30	140	139	49.8	3	136	132 Western porcupine grass 5 Mountain bunch-grass 3 Short-awned brome-grass	3	Vigorous	
31	133	353	72.6	11	342	116 Western porcupine grass 17 Mountain bunch-grass	4	Severely trampled; quadrat on public sheep trail.	Seedlings previously weakened through competition and drought.
32	34	32	48.4	3	29	30 Short-awned brome-grass 3 Western porcupine grass 1 Mountain bunch-grass	3	Vigorous	
33	60	28	31.8	6	22	59 Western porcupine grass 1 Mountain bunch-grass	1	....do	
34	45	9	16.6	7	2	40 Smooth wild rye 3 Mountain bunch-grass 2 Western porcupine grass	8	Smooth wild rye has made unusual height growth. Vigorous.	Had escaped close grazing.
35	37	36	49.3	1	35	30 Smooth wild rye 7 Western porcupine grass	3	Fair; showed effects of trampling.	
36	66	35	34.6	11	24	61 Smooth wild rye 3 Western porcupine grass 2 Mountain bunch-grass	7	Good	
37	92	154	62.6	5	149	144 Western porcupine grass 4 Mountain bunch-grass 1 Sickle sedge	4	Many seedlings eaten off and partly pulled out of ground.	
38	29	8	21.6	4	4	27 Smooth wild rye 2 Little bluegrass	.....	Medium strong	
39	137	80	36.8	.....	80	134 Western porcupine grass 3 Mountain bunch-grass	.....	Vigorous	

[a] Of the seedlings whose recovery from grazing, drought, or other causes was doubtful, none were included in the number composing the "total number of seedlings remaining," as shown in column 2 of this table.

TABLE XI.—*Record of native forage seedling stand at high elevations after September 1, after grazing*—Continued

Quadrat No.	Total number of seedlings remaining.	Total loss.	Percentage lost.	Number found dead.	Number not accounted for.	Number of plants of remaining seedling species.	Number whose recovery seemed doubtful.	Condition of remaining seedling stand at time of recount.	Remarks.
30	118	17	12.5	4	13	101 Little bluegrass 8 Slender hair-grass 1 Yarrow	14	A few weak, most of them vigorous.	Greatest injury to seedlings due to early drought period.
31	48	33	40.3	7	26	31 Western porcupine grass 7 Little bluegrass 4 Blue beardtongue 3 Smooth wild rye	5	Vigorous; had produced unusual growth in height.	
32	122	36	16.2	13	17	138 Little bluegrass 9 Smooth wild rye 6 Yarrow 2 Mountain bunch-grass	8	Vigorous	
33	70	24	25.5	3	21	57 Western porcupine grass 5 Little bluegrass 5 Sickle sedge 3 Mountain bunch-grass		A few doubtful of recovery; others vigorous	
34	148	56	27.4	15	41	128 Smooth wild rye 11 Yarrow 9 Mountain dandelion	2	Vigorous	
35	171	139	46.7	12	127	171 Western porcupine grass		Good	
36	29	84	74.9	4	30	9 Mountain bunch grass 7 Mountain dandelion 4 Yarrow	4	Medium	Seedlings weakened through the gullying of the soil
37	51	38	42.7	4	34	44 Western porcupine grass 6 Alpine redtop 1 Smooth wild rye	6	Vigorous	
38	31	3	8.8	.....	3	29 Mountain bunch grass 2 Yarrow	6	.....do	
39	165	122	42.5	6	116	163 Western porcupine grass 2 Yarrow	4	.....do	
40	44	73	34.3	9	64	29 Smooth wild rye 14 Yarrow 1 Mountain bunch grass	5	do	Some seedlings destroyed by gopher mounds
41	41	124	74.7	.....	124	36 Western porcupine grass 5 Mountain bunch-grass	10	Medium	Sheep trail through quadrat responsible for heavy loss.
42	15	11	42.3	2	9	15 Mountain bunch-grass	1	Vigorous	
43	22	16	41.9	4	12	21 Mountain bunch-grass 1 Sickle sedge		Medium; showed effects of drought	
44	5	21	80.7		21	4 Mountain bunch grass 1 Sickle sedge		Excellent	

55											
56		91	230	71.6	18	93	92	Western porcupine grass	5	Very poor	Drought had severely weakened seedlings early in season.
							13	Mountain bunch-grass			
		15	38	34.8	1	16	3	Western porcupine grass		Good	
57		9	23	71.8	1	11	9	Mountain bunch-grass		Vigorous	
58		14	11	68.8	1	28	20	Mountain bunch-grass		do	
							4	Western porcupine grass			
							1	Mountain bunch grass			
59		10	17	69.9	1	13	2	Western porcupine grass	1	Medium strong	
							1	Sickle sedge			
60		17	13	43.3	1	13	17	Mountain bunch-grass	1	Vigorous	Soil firm and remaining seedlings little injured by trampling.
61		18	11	37.9	3	7	18	Mountain bunch grass	3	Vigorous; have made unusual growth in height.	
62		11	24	68.5	1	21	11	Mountain bunch grass	1	Vigorous	Narrow trail made through quadrat, killing all seedlings in its path.
	Average	80.8	74.5	48.2							

It will be seen that both before and after grazing the more abundant seedlings were of mountain bunch-grass, smooth wild rye, short-awned brome-grass, sickle sedge, little bluegrass, and western porcupine grass. Table X shows that before grazing there was an average for all quadrats of 155.3 seedlings per square meter. After grazing (Table XI) this was reduced to 80.5 seedlings, a loss of 48.2 per cent. A comparison of the height and root development of the same seedling species at medium and high elevations discloses the fact that the shorter and later growing season of the high range had not been conducive to the rapid development made by the seedlings in the lower and warmer, though drier, situations. Though the high ranges were grazed much more moderately, the loss was practically the same as on the lower ones.

The factors responsible for the heaviest loss of seedlings through grazing were (1) superabundance of soil moisture, (2) lack of soil moisture, (3) abnormally dense seedling stands, and (4) irregular topography.

In wet situations the roots did not penetrate as deeply as in the more compact and drier soils, and so were more easily disturbed. Although in some cases the seedlings recovered, the loss on the moist soils was relatively large, in fact occasionally five times that on the dry soils.

In excessively dry situations the loss from grazing was often serious, owing to the relatively weak growth made by the seedlings and their poor recuperative power. In some places practically the entire seedling stand was destroyed. Doubtless many of the seedlings whose destruction was charged to grazing would have perished in any event from drought, though the stand as a whole was not affected by this factor. The lack of vigor in individual plants where the stand was unusually dense often caused a heavy loss. Quadrats with from 250 to 500 seedlings almost invariably suffered more than the contiguous plots which carried a sparser stand. In general, it may be said that more than 200 seedlings to a square meter is a heavier stand than most situations can support permanently. Competition is most severe between plants of the same species, since each plant makes the same demand upon the habitat. Where a dense stand occurred it was usually of a single species.

The loss from trampling was much more severe on steep slopes than on level situations. This was largely the result of the coarser texture of the soils on steep slopes and of the greater extent to which they are shifted by grazing. Moreover, because of the lack of soil moisture on many of the steeper slopes, the plants growing there are generally less vigorous than those growing in more level places.

TIME AND INTENSITY OF GRAZING AFTER THE FIRST YEAR

Though the information here presented shows that the range upon which deferred grazing was practiced suffered heavy loss of seedlings when moderately grazed, it should not be concluded that in order to

insure permanent improvement grazing must be suspended from the time the first seed crop is produced until the seedlings become established. Notwithstanding the fact that half the stand in existence in the autumn is likely to be eliminated by grazing, the planting of an additional seed crop will, as a rule, fully offset this loss.

The time at which the seed crop of the established vegetation reaches maturity, marking the approximate limit of growth and occurring in the region studied about September 1, is the beginning of the period when the range may be grazed with the least injury to forage seedlings. During the four weeks prior to this period the root system almost doubles its growth and strength.

On account of the much more elaborately developed root system at the end of the second year of the seedlings' growth, the loss through grazing at that period perceptibly lessens. Even then, however, the range should not be grazed prior to the maturity of the seed crop. Restriction of grazing to the period following seed maturity will give both the 1- and 2-year-old plants sufficient protection to insure the restocking of the range.

To sum up the conclusions regarding deferred grazing, it may be said that the system has proved highly successful wherever an adequate seed crop was produced. Its advantages over yearlong grazing and yearlong protection are (1) the restoration and maintenance of the vegetation without the loss of the forage crop in any year, (2) the planting of the seed, and (3) the removal of the vegetation itself, thus minimizing the fire danger from an accumulation of inflammable material.

Deferred grazing has all the advantages of complete protection, so far as the rejuvenation of the weakened plants is concerned; and, if overstocking and abusive management are guarded against, the system will work no material injury to forest reproduction or watersheds. It is believed, therefore, that the principles of deferred grazing, with whatever modifications are necessary to meet local conditions, should be applied to the management of all ranges.

APPLICATION OF DEFERRED GRAZING SYSTEM TO RANGE MANAGEMENT

WHERE APPLICABLE

If grazing lands are to be fully revegetated within a reasonable time, the range lands must, of course, support at the outset at least a sparse stand of the species valuable for grazing and revegetation purposes. In the Wallowa Mountains, where mountain bunch-grass constitutes the predominating herbage, a satisfactory seed crop and subsequent seedling stand were secured where the original tussocks stood as far apart as 6 feet. Where grazing has been so severe as to destroy the major portion of the original vegetation, the remaining plants may not produce

viable seed until after they have regained their lost vigor, a matter of one or two seasons. On other sparsely vegetated lands, however, a stand of from 15 to 30 seedlings per meter, which is a satisfactory density on most soils, has been secured after the first year of deferred grazing.

The benefits of deferred grazing are not confined to areas which support plants of strong seed habits or those where the climate is particularly favorable to growth. Though on areas near and above timber line, where most of the forage plants reproduce vegetatively instead of by seed, deferred grazing does not tend to augment vegetation as it does on areas where the plants reproduce by the latter method, it does result in a permanent increase in vigor of the range plants and so promotes vegetative reproduction, which otherwise would be held in check by the premature removal of the herbage each season. In short, given a sufficient number of the original plants, deferred grazing is applicable wherever the vegetation is palatable after the seed crop has ripened and where water facilities will permit the range to be used in the autumn.

Before the deferred grazing system was thoroughly tried out, certain stockmen maintained that after seed maturity the palatability and nutritiousness of the herbage would be low and therefore that the season's forage crop would not only be wasted, but stockmen might be induced to keep their animals in the mountains until so late in the season that on account of the resultant loss of weight they would not be able to market them direct from the summer range.

To determine definitely the nutritive value of the forage after seed maturity, chemical analyses were made of the foliage of mountain bunch-grass, first, when the flower stalks were being produced, and again, at the time the seed ripened. The average of the tests showed that the young growing plant is 27.21 per cent richer in ether extract (fat) than the mature plant, while the latter slightly exceeds the former in protein (nitrogen). The mature plant also contains more crude fiber, but since the flower stalks are not consumed after the seeds are ripened that part of each specimen was eliminated from the tests. In comparison with timothy hay, mature mountain bunch-grass contains 94.39 per cent more protein, practically the same amount of ether extract, and 50.45 per cent less crude fiber, the last-named material being practically indigestible.

Nearly all the leading range plants, particularly the grasses, are grazed during the autumn with relish. It can not be said, however, that they are eaten with the same gusto after seed maturity as when they are growing vigorously. It was found that the first time a band of sheep passed over a matured range of medium density only about half of the forage crop was grazed off. Not until the range was grazed a second or third time was the crop entirely consumed. The vegetation on similar ranges grazed a month earlier was in most cases entirely con-

sumed the first time the stock passed over it. On ranges grazed after seed maturity the naked flower stalks, rising from leafless tufts of bunchgrass, remained after the stock had passed over them, but on ranges grazed when the forage was succulent and tender no flower stalks were visible after the passage of the stock. No appreciable amount of herbage remained on either area.

Sheep from several allotments where deferred grazing was practiced made fully as good progress as other sheep in allotments not handled under deferred grazing. By the time the seed has ripened, the milk flow of the ewes is nominal, and though it may decrease slightly when the animals are placed on the semi-air-cured forage, the lambs by this time are 4 or 5 months old, and milk is secondary to the nourishment secured through cropping.

Deferred grazing does not materially change the character of the forage on mountain ranges after seed maturity, because by this time succulent forage everywhere has been reduced to a minimum, leaving only the air-cured plants and on open grazing lands a small amount of second growth. By protecting part of the range until the last few weeks of the grazing season there is the possible advantage of having a reserve supply of solid feed upon which to harden the stock prior to the drive to market or to winter range.

The water facilities of the range may be an important consideration in determining whether or not to adopt the deferred grazing system. Regardless of the palatability of the forage, deferred grazing can not be carried out unless there is an adequate supply of water. On many ranges the water facilities may be improved by the construction of dams, the development and protection of springs, and even by digging wells and building windmills. Springs and small mountain streams are often replenished by the autumn rains.

SELECTION OF LANDS

The amount of range needed for grazing under the deferred system depends upon (1) the time at which the seed of the important forage plants matures and (2) the portion of the grazing season remaining after seed maturity. In the mountains of northeastern Oregon one-fifth of the grazing season remains after seed maturity. Accordingly, one-fifth of the carrying capacity, but not necessarily of the total acreage, of each grazing allotment may be reserved annually for purposes of revegetation. The lower the elevation the earlier, of course, does the seed mature and the greater the proportion of range which must be reserved for deferred grazing. Since the lands are usually grazed by camps, the carrying capacity of which is well known, the user will have no difficulty in determining what proportion of the range should be reserved.

MANAGEMENT DURING THE REVEGATION PERIOD

Once the area in need of revegetation has been selected, no stock should be allowed to graze on it until after the seed has ripened. Efforts should then be made to have the stock pass at least once over the entire area reserved, in order thoroughly to plant the seed.

In the second year of deferred grazing if a reasonably dense stand of forage seedlings has been secured, abusive herding must be avoided. While it may do no apparent harm, so far as future seed crops are concerned, to fully utilize the forage in the fall after the first year of protection, the loss of seedlings, even when the range is only moderately grazed, amounts to about 50 per cent. Close grazing and carelessness in permitting the stock to bunch and trail must necessarily increase this loss. Therefore, while close grazing after seed maturity the second year may result in increasing the forage seedling stand the following season, such an increase could only be temporary, since the practice causes severe loss among the seedlings already in existence. Moderate grazing after seed maturity also, of course, results in the destruction of a large number of seedlings, but the double advantage of utilizing the forage and planting an additional seed crop readily offsets this loss. Moderate grazing should be practiced in the second and subsequent seasons until the plants have reached full maturity and are permanently established. In the case of perennial plants this period is three years.

When the area selected has been thoroughly reseeded and the plants permanently established, another area in need of reseeding should be selected. This system should not come to an end when the range has been completely reseeded, but should be kept up in order to thoroughly maintain the vigor of the vegetation and allow for an occasional seed crop.

During the season of 1912 deferred grazing was in effect on 10 allotments in various portions of the Wallowa National Forest. In every case the carrying capacity of the range has increased materially, and the best interests of the stock industry seem to call for the adoption of the system generally.

SUMMARY

(1) Normally the spring growth of forage plants begins in the Hudsonian zone about June 25. For each 1,000 feet decrease in elevation this period comes approximately seven days earlier.

(2) In the Wallowa Mountains the flower stalks are produced approximately between July 15 and August 10, while the seed matures between August 15 and September 1.

(3) Even under the most favorable conditions the viability of the seed on summer ranges is relatively low.

(4) Removal of the herbage year after year during the early part of the growing season weakens the plants, delays the resumption of growth,

advances the time of maturity, and decreases the seed production and the fertility of the seed.

(5) Grazing after seed maturity in no way interferes with flower-stalk production. As much fertile seed is produced as where the vegetation is protected from grazing during the whole of the year.

(6) Germination of the seed and establishment of seedlings depend largely upon the thoroughness with which the seed is planted. In the case of practically all perennial forage species the soil must be stirred after the seed is dropped if there is to be permanent reproduction.

(7) Even after a fertile seed crop has been planted there is a relatively heavy loss of seedlings as a result of soil heaving. After the first season, however, the loss due to climatic conditions is negligible.

(8) When 3 years old, perennial plants usually produce flower stalks and mature fertile seed.

(9) Under the practice of yearlong or season-long grazing both the growth of the plants and seed production are seriously interfered with. A range so used, when stocked to its full capacity, finally becomes denuded.

(10) Yearlong protection of the range favors plant growth and seed production, but does not insure the planting of the seed. Moreover, it is impracticable, because of the entire loss of the forage crop and the fire danger resulting from the accumulation of inflammable material.

(11) Deferred grazing insures the planting of the seed crop and the permanent establishment of seedling plants without sacrificing the season's forage or establishing a fire hazard.

(12) Deferred grazing can be applied wherever the vegetation remains palatable after seed maturity and produces a seed crop, provided ample water facilities for stock exist or may be developed.

(13) The proportion of the range which should be set aside for deferred grazing is determined by the time of year the seed matures. In the Wallowa Mountains one-fifth of the summer grazing season remains after the seed has ripened, and hence one-fifth of each range allotment may be grazed after that date.

(14) The distribution of water and the extent of overgrazing will chiefly determine the area upon which grazing should be first deferred.

(15) After the first area selected has been revegetated it may be grazed at the usual time and another area set aside for deferred grazing. This plan of rotation from one area to another should be continued, even after the entire range has been revegetated, in order to maintain the vigor of the forage plants and to allow the production of an occasional seed crop.

PLATE XII

Fig. 1.—View of the lower grazing lands in the Wallowa National Forest. The timber is western yellow pine. The exposed situations are covered with a dense growth of big bunch-grass (*Agropyron spicatum*).

Fig. 2.—Characteristic open stand of western yellow pine and dense cover of herbaceous vegetation, mainly pine-grass (*Calamagrostis pubescens*), Wallowa National Forest. Transition zone (yellow-pine association).

Fig. 3.—A burned-over area of lodgepole pine, with characteristic dense sapling stand.

1
3
2

1

2

3

PLATE XIII

Fig. 1.—Dense stand of lodgepole pine, with undergrowth of red huckleberry (*Vaccinium scoparium*). Canadian zone (lodgepole-pine association).

Fig. 2.—A flat eminence in the Hudsonian zone, showing the characteristic clumped growth of whitebark pine and Alpine fir. The glade land was formerly densely vegetated with mountain bunch-grass.

Fig. 3.—Irregular topography of the upper grazing lands. On northerly exposures snow often remains until August. Photographed on July 25, 1908. Hudsonian zone (whitebark-pine association).

PLATE XIV

Fig. 1.—Arctic-Alpine and upper subalpine region, where forage is sparse, due to poor soil, short growing season, and unfavorable climate.

Fig. 2.—Mountain range lands prior to the beginning of growth and germination. Photographed on June 21, 1908.

Fig. 3.—Same view as shown in figure 2, but more in detail, showing the condition eight days later (June 30). The conspicuous plant in the foreground is spring beauty (*Claytonia lanceolata*), which closely follows the recession of the snow and announces the earliest approach of spring.

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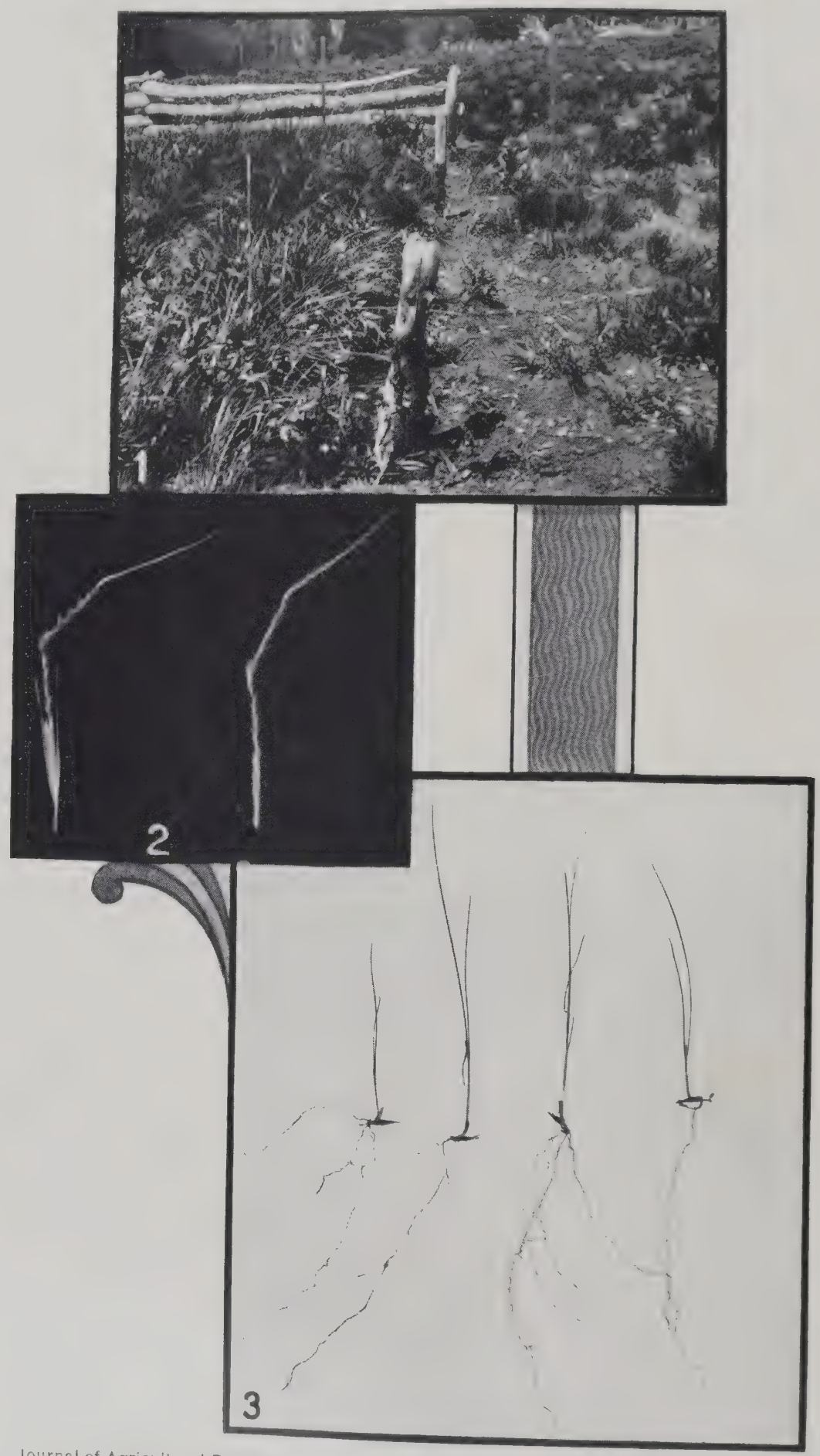
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PLATE XV

Fig. 1.—Contrast in the progress of the flower stalk production of mountain bunch-grass on portion of range which has been completely closed to grazing for a period of three successive years and on range which has been subject to continued early grazing. Section of fence temporarily removed. Photographed on July 7, 1909, before grazing.

Fig. 2.—Western porcupine grass (*Stipa occidentalis*), showing empty glumes and floret with the scale and its awned projection to the left; to the right the floret with glumes removed, showing the sharp-pointed, slightly-curved seed tip. Natural size.

Fig. 3.—Average development of the root system and aerial portion of mountain bunch-grass at end of the first growing season. Natural size.

PLATE XVI

Mountain bunch-grass, showing root development and aerial growth at the end of the second season. Natural size.

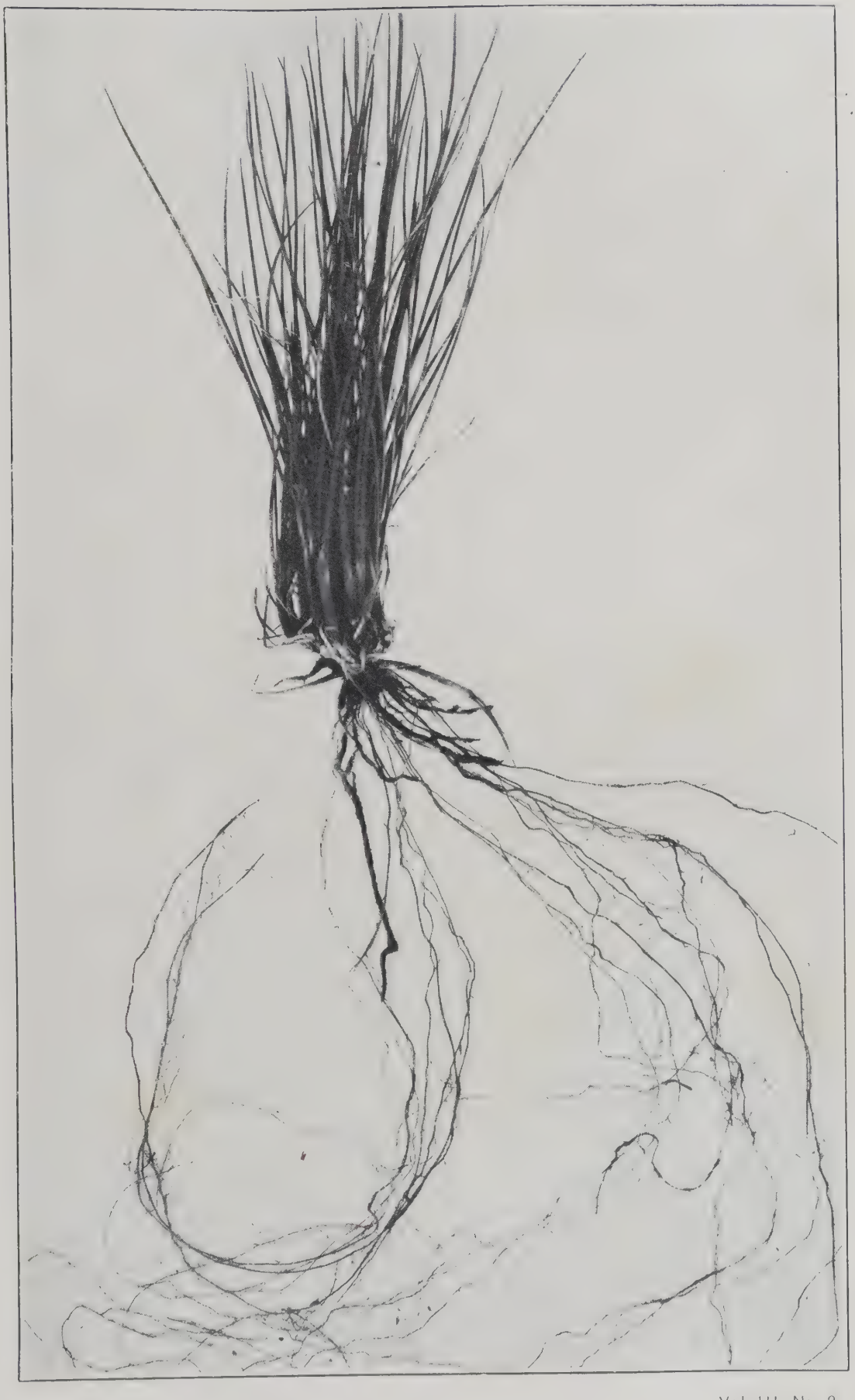

PLATE XVII

Mountain bunch-grass in the spring of the third year of growth just before producing flower stalks, showing the natural position and length of the elaborate root development and aerial growth. Natural size.

PLATE XVIII

Mountain bunch-grass at the end of the third year, showing three flower stalks and inflorescence. Natural size.

PLATE XIX

Sickle sedge (*Carex umbellata brevirostris*), showing offshoots from the root-stocks and flower stalks with fruit in the process of development. This is an aggressive but unpalatable plant which is reproducing abundantly on overgrazed ranges under the prevailing grazing practice. Natural size.

PLATE XX

Fig. 1.—Station 4 on Stanley Range as it appeared on July 12, 1907. Elevation, 7,400 feet.

Fig. 2.—View of station 4 on July 15, 1909, after two years' protection from grazing animals. The apparent increase in forage is due to luxuriant growth of the vegetation in existence when stock was eliminated and to slight vegetative increase in the grass tufts. No perennial seedlings were found on this area.

Fig. 3.—View of quadrat No. 1, established on July 10, 1907. Annual weeds constitute the predominant vegetation. Elevation, 3,000 feet.

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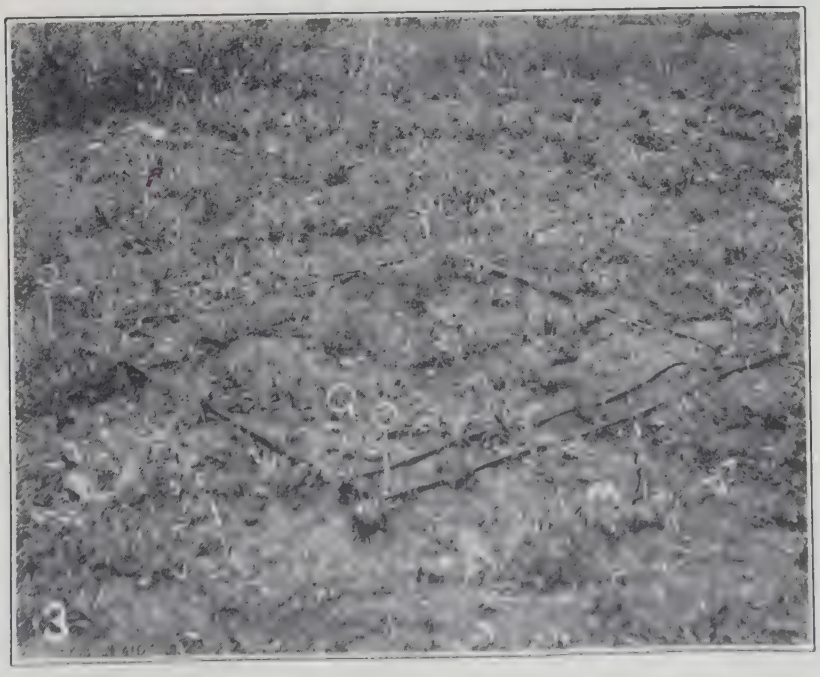
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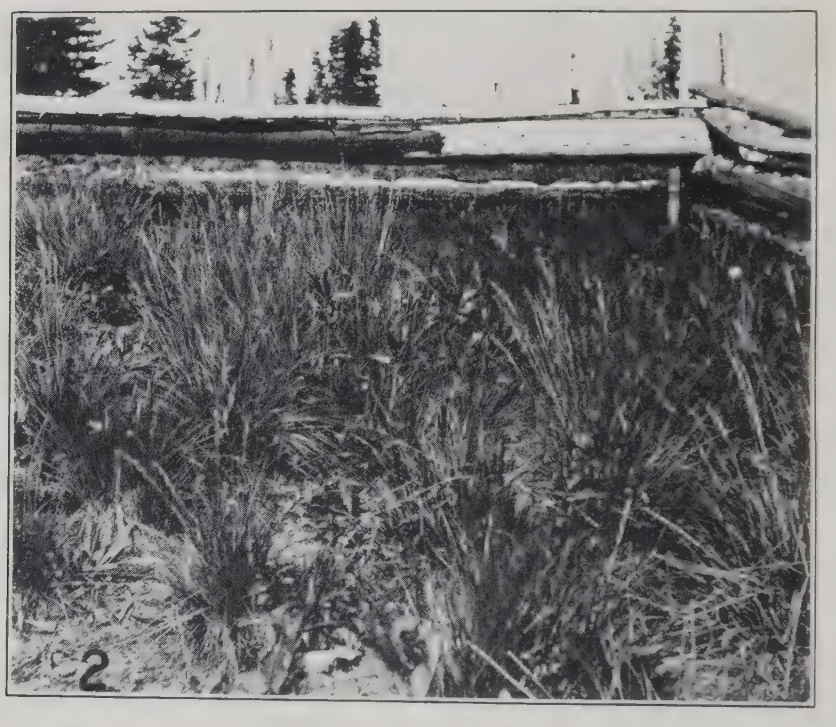
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PLATE XXI

Fig. 1.—Quadrat 1, as it appeared on July 16, 1909. Soft cheat occupies four-fifths of the quadrat, the balance being composed mainly of mountain June-grass, geranium, and yarrow.

Fig. 2.—Area of mountain bunch-grass closed to grazing animals on July 8, 1907. Photographed July 7, 1909. Stanley Range, elevation approximately 7,400 feet.

Fig. 3.—View of open range contiguous to area shown in figure 2. Photographed on July 7, 1909.

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PLATE XXII

View of plot in the Transition (yellow-pine) zone which has been protected from grazing animals for three successive years, showing contrast in carrying capacity with contiguous open range. The increase in stand is due almost entirely to the reproduction of annual plants.

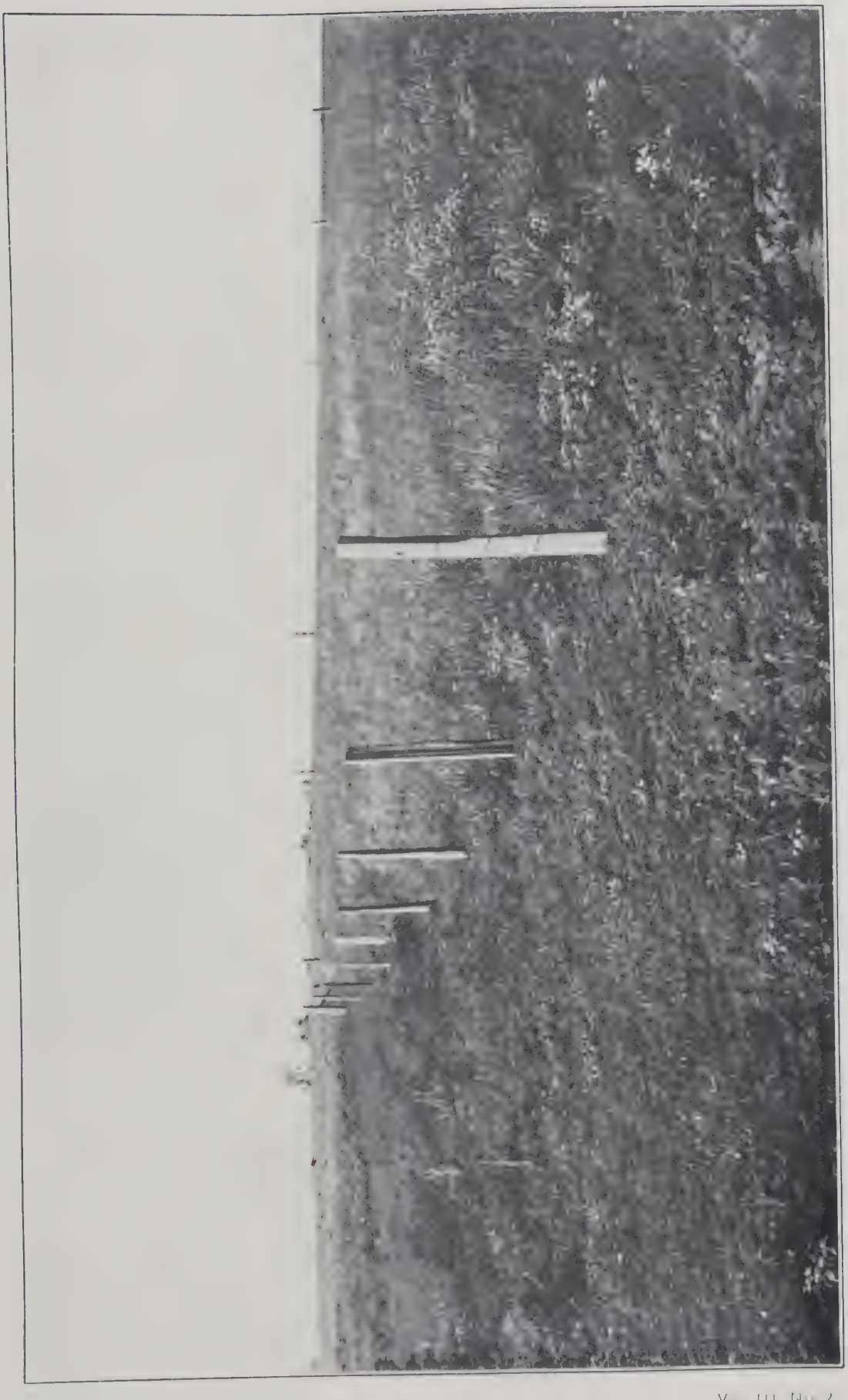

PLATE XXIII

Fig. 1.—View of portion of allotment at medium elevation where the destruction of forage seedlings due to grazing and trampling was studied.

Fig. 2.—Dense stand of smooth wild rye (*Elymus glaucus*) and short-awned bromegrass (*Bromus marginatus*) seedlings. These species were fully 4 inches tall by August 15 and were invariably grazed by sheep on areas comparatively free from other vegetation.

PECAN ROSETTE

By W. A. ORTON, *Pathologist in Charge, Cotton and Truck Disease and Sugar-Plant Investigations, and* FREDERICK V. RAND,[1] *Assistant Pathologist, Laboratory of Plant Pathology, Bureau of Plant Industry*

HISTORY AND DISTRIBUTION

Rosette has been rather generally recognized by growers as a serious disease almost from the inception of commercial pecan orcharding. As early as 1902 requests came to the United States Department of Agriculture for an investigation into the causes of the disease and possible methods of control. The work was at once undertaken by the senior author and carried on for about four years in connection with other

FIG. 1.—Map showing the known distribution of pecan rosette in the United States.

work in the Southern States, but between 1906 and 1910 little attention was paid to the disease. Since 1910, and more particularly during the seasons of 1912 and 1913, the experimentation has been continued by the junior author.

The disease is well distributed over the pecan-growing territory from Texas to the Atlantic coast and from Florida to Virginia. (See fig. 1.) It has been definitely seen by one or the other of the authors at Whittier, Cal.; San Antonio, Boerne, Waring, Kerrville, San Saba, Waco, Austin, McKinney, Tex.; New Orleans, La.; Ocean Springs, Miss.; Atlanta,

[1] The work of the junior author was carried out while he was employed as scientific assistant in the Office of Fruit-Disease Investigations, Bureau of Plant Industry.

Statesboro, Albany, De Witt, Baconton, Thomasville, Cairo, Valdosta, and Blackshear, Ga.; Belleview, Palatka, Sisco, Gainesville, St. Augustine, Jacksonville, McClenny, Glen St. Mary, Alachua, Lake City, Monticello, Newport, and Tallahassee, Fla.; Mt. Pleasant, Denmark, Bamberg, Greenwood, Blackshear, Orangeburg, St. Matthews, Fort Motte, Cameron, Sumter, Summerton, and James Island, S. C.; Durham, N. C.; and at Eastville, Va. Besides personal observations at the places above enumerated, specimens of pecans (*Carya illinoensis*) showing undoubted symptoms of rosette have been received from a much wider territory including Arizona, Tennessee, and other States. Similar symptoms have been observed by the authors upon other species of hickory, notably the mockernut (*Carya alba* (L.) K. Koch.), and the pignut (*C. glabra* (Mill.) Spach.), also upon the butternut (*Juglans cinerea* L.), the rock walnut of Texas (*Juglans rupestris* Engelm.), the hackberry (*Celtis occidentalis* L.), and the common locust (*Robinia pseudacacia L.*).

Furthermore, pecan rosette does not appear to be limited to any particular soil type, topography, or season. We have noted many distinct and undoubted cases in the deep sand of the Florida Coastal Plain with the water table at 3 to 3½ feet from the surface, farther inland in deep sand or sandy loam with the water table varying from 2 to 10 feet, in sand or sandy loam underlain by yellow, red, or white clay at depths varying from a few inches to several feet and with a varying water table, in the clay or sandy clay of washed-out hillsides, in the river bottom and alluvial soils of Louisiana and Texas, in the black upland soils of Texas, in cultivated and uncultivated land, with and without fertilization, in extremely rich and extremely poor soils, and in wet and dry seasons. In fact, for the localities personally investigated, swamp land has presented the only location so far entirely exempt. It is true that wherever the soil tends to be water-soaked through a considerable portion of the growing season the pecan presents an unhealthy appearance through its failure to make proper growth and through the sickly yellow appearance of the leaves. Under such conditions the tree usually dies sooner or later. The symptoms, however, bear so little resemblance to those of rosette that even the most casual observer will not confuse the two diseases.

SYMPTOMS AND VIRULENCE OF PECAN ROSETTE

Pecan rosette first makes itself evident through the putting out of undersized, more or less crinkled, and yellow-mottled leaves (Pl. XXIV, figs. 1 and 2), particularly at the ends of the branches. The veins tend to stand out prominently, giving a roughened appearance to the leaf blade, and the light-green or yellowish areas which give the leaf its mottled appearance occur between the veins. In these light-colored parts the tissues are thinner and less fully developed than in the normal leaf, and later in the season they frequently become dark reddish brown and dead.

In many cases the intervascular tissue here and there fails to develop at all, so that the lamina is dotted with smooth-margined holes suggesting insect perforations which have subsequently healed over (Pl. XXV). These first symptoms may occur over the whole tree at once, but often one or more branches may be affected for several months before the whole tree appears involved. At this stage the foliage as a whole often presents a rusty appearance. The diseased branches usually fail to reach their normal length, so that the leaves are clustered together on a shortened axis, giving a bunched appearance to the group which led the senior author, about 1902, to apply the term "rosette" as an appropriate name for the disease (Pl. XXVI; cf. fig. 1, rosetted shoot, with fig. 2, normal shoot). Nuts are frequently borne and carried to maturity on these branches.

In some cases the disease goes no farther. The trees may continue in this way for several seasons, or they may recover completely after showing the early symptoms for one or more years. However, in a well-defined case where the symptoms are general over the greater part of the tree, the affected branches begin to die back from the tip during the latter part of the first season or later (Pls. XXVII and XXVIII). At first brownish spots and streaks appear in the green bark, and these dead areas increase in size until the whole end of the twig or branch dies. While death appears to start in the green bark, the cambium soon becomes affected and the wood and pith are usually discolored. This dying back or "staghorn" stage is followed during the same or the following season by the development of numerous lateral shoots from dormant or adventitious buds. In young vigorous trees these first shoots of the season are usually large and succulent, and the leaves are dark green and above the normal in size. In all probability this effect is physiologically equivalent to the effect of severe pruning. Toward the middle of the season, however, the typical yellow-mottled color appears and the later-developed leaves are more or less crimped and roughened, as well as below the normal in size. Dormant axial buds of one or two series may develop into abortive shoots, and toward the end of the season clusters of short or spindling branches usually put out from adventitious or dormant buds farther back on the branches or on the main trunk. The leaves in these cases are much reduced in size and may appear as a mere skeleton with ragged edges.

This process goes on from year to year. The growth of the tree is checked, and these abnormal clusters of branches are formed only to die back each season and be followed by others. Thus a well-marked case of several years' standing presents a characteristically gnarled and forlorn appearance (Pl. XXVIII, fig. 3). Rosette in all its forms occurs in trees from seedling and budded or grafted nursery stock to trees of long-established maturity, a hundred or more feet in height, and it is one of the worst diseases known to affect pecans.

PRUNING EXPERIMENTS

If the rosette were of a parasitic nature, it seemed entirely possible that a severe pruning out of the diseased parts or at least a cutting back to the stump might entirely eliminate the disease. To test out this proposition, 10 distinctly rosetted trees in the orchard of Mr. J. B. Wight, Cairo, Ga., were severely pruned and 5 similarly diseased trees were cut off at the ground and allowed to send up sprouts from the stump. This work was done in the winter of 1902–3, and observations the following midsummer showed the new growth in all the trees to be distinctly rosetted (Pl. XXVIII, fig. 2).

In like manner three 7-year-old trees were severely cut back, or "dehorned," and five other badly diseased trees were cut back to a stub 18 inches high. This work was carried out in February, 1912, in the orchard of Mr. G. W. Saxon, Tallahassee, Fla. The following spring most of the new growth was vigorous and the leaves were dark green and normal in appearance. Toward midseason, however, the leaves began to appear yellow mottled and those most recently developed were undersized; before the end of the season every tree and nearly every shoot was badly affected with rosette.

In the summer of 1911 three badly diseased trees belonging to the Standard Pecan Co,, Monticello, Fla., were cut back to the trunk. The following midsummer all the new growth was rosetted as badly as before cutting back.

Further observations have been made upon the effect of severe pruning and cutting back in orchards at Belleview, St. Augustine, Monticello, and Tallahassee, Fla ; Thomasville, Baconton, and Albany, Ga.; and at Orangeburg, S. C. In all cases the same negative results have occurred. Usually in vigorous trees the new growth appears healthy, as in the case of rosetted trees severely cut back by the disease itself; but before the end of the summer or at least by the next season the rosette again appears. The disease was in no case eliminated by pruning.

TRANSPLANTING EXPERIMENTS

In order to determine whether the cause of the disease was to be sought in the tree itself or in the soil, several transplanting and germination tests were carried out.

In December, 1902, 8 badly rosetted trees were dug up from the J. B. Wight orchard at Cairo, Ga., and healthy seedling nursery trees were immediately set in the holes. At the same time 41 nursery trees were set in vacant places where no trees of any kind had been growing for one or more years. The following August, 1 tree out of the first group and 8 out of the second were dead, probably from effects of transplanting. All the remaining trees were apparently in a normal condition.

Of the remaining 7 trees in the first group, 4 continued healthy for two seasons and were dug up. One was badly rosetted the following season, 1 showed a slight trace of the disease at two years and was dug up, and the last tree, which was normal at the time of observation in 1903, 1904, and 1912, showed a distinct case of rosette in 1913. Of the 33 remaining trees of the second group, 16 remained normal through two years, and were cut out, 5 were normal at the time of all observations, while 12 at one time or another showed distinct symptoms of rosette. It will be noted that the percentage of trees contracting the disease did not differ greatly in the two cases.

In the same orchard, during the fall and early winter of 1904–5, 35 healthy trees, comprising 11 varieties, were set in holes occupied by rosetted trees within one year. Likewise, 11 trees, comprising 7 varieties, were set after healthy trees had been removed or in places previously unoccupied. Observations in 1912 and 1913 showed 33 trees of the first group with pecan rosette and only 2 normal. Of the second group, 6 contracted rosette, while 5 were normal in appearance.

Similarly in February, 1908, 27 trees of 7 varieties were set after rosetted trees, and 4 trees of 3 varieties in vacant places or after healthy trees. Observations in 1912 and 1913 showed 24 trees of the first group to be rosetted, and 3 healthy. In the second group 2 were rosetted, while 2 were healthy.

In the winter of 1904–5, 10 rosetted nursery seedlings at Cairo, Ga., were transplanted to a part of the same field previously unoccupied by pecan or other hickory trees. Observations during October, 1905, showed 6 trees apparently normal, 3 with symptoms of rosette on the older leaves, but with the later growth normal in appearance, and 1 with traces of rosette. The following August, 9 trees appeared healthy, and 1 presented a doubtful case of rosette. In August, 1907, all 10 trees were apparently normal. In February, 1908, 2 trees which had died from unknown causes were replaced with healthy trees of the Stuart variety. The next observation, made in September, 1912, showed 6 distinct cases of rosette (including the 2 Stuart pecan trees above mentioned), 2 trees with doubtful symptoms of rosette, and 2 normal. The last note, made in August, 1913, showed 5 distinct cases of rosette, the same 2 doubtful cases, and 3 normal trees. Four of the rosetted trees were much improved in appearance over that of the preceding season.

Forty-three rosetted nursery trees at Glen St. Mary, Fla., were transplanted from the nursery row (March, 1907), where the water table was about 18 inches below the surface, to another part of the place where the soil was a loamy sand underlain by clay, with the water table at a considerable distance below the surface. Owing probably to the late spring transplanting, 18 of the trees died without putting out leaves. Of the remaining trees (October, 1907) 18 showed distinct symptoms of

rosette, 3 had doubtful traces, while 4 were normal. During the following winter part of the vacant places were filled from the nursery, making 38 trees in all. No further observations were made until the summer of 1910, at which time no traces of the disease were apparent. In August, 1912, 18 trees were normal, 12 were distinctly rosetted, while 6 showed traces of the disease. The following August, 31 trees were normal, 2 were distinctly rosetted, and 3 showed traces of the disease. Throughout the experiment the trees received no pruning, and little attention of any kind save an occasional cultivation and moderate applications of a complete commercial fertilizer.

In December, 1903, 6 nursery trees showing symptoms of rosette were taken up at Dewitt, Ga., and sent by Mr. Herbert C. White to Washington, D. C. During the latter part of November, 1904, a like number of rosetted nursery trees were sent by Mr. J. B. Wight from Cairo, Ga. Upon receipt these trees were potted in garden soil and placed in one of the greenhouses of the Department of Agriculture. All the trees lived, but observations up to January, 1907, gave no evidence of rosette in any of them. At this time 4 were set out at Takoma Park, D. C., 4 at Glen St. Mary, Fla., along with the trees described in the preceding experiment, and the remaining 4 were left in the greenhouse. The Takoma Park trees died from other causes after the second winter, but showed no more rosette. The 4 trees set at Glen St. Mary, Fla., were healthy during 1910 and 1911. In August, 1912, 1 tree showed a trace of rosette, but the following season all 4 were healthy. The greenhouse trees remained healthy until destroyed the next year to close the experiment.

In the winter of 1912, 5 Stuart nursery trees which had reached the staghorn stage of rosette were sent by Mr. H. K. Miller from Monticello, Fla., to Washington, D. C., where they were potted in garden soil and placed in one of the Department greenhouses. The trees were rather large for potting, and therefore both roots and tops were severely pruned. Probably as a result of this severe treatment, together with the almost entire absence of lateral roots, the tops of all 5 trees died, but the following season 3 sent up sprouts from the crown. These shoots have made a perfectly normal growth for three seasons and have at no time shown the faintest traces of rosette.

In 1910 a badly rosetted Stuart pecan tree in the orchard of Dr. R. B. Garnett, at St. Augustine, Fla., was taken up by the owner and reset in another part of the place. The following winter 5 badly diseased young orchard trees were taken up and reset about a quarter of a mile distant. Observation by the junior author in August, 1912, showed the Stuart tree to be entirely recovered. Three out of the second group were entirely normal in appearance, while two still had symptoms of the disease.

It will be noted from the results of these experiments in transplanting that of the healthy trees set after rosetted trees nearly all subsequently contracted the disease, while of those set after healthy trees only about half subsequently showed symptoms of the disease. This would point toward the conclusion that some relation exists between pecan rosette and the soil, either directly through the soil itself or through its previous infection by rosetted trees.

Furthermore, it has been shown that of rosetted trees set after healthy trees in the same locality or replanted in entirely different situations, a very high percentage of the trees and often all recovered. This would tend to indicate that the soil relation is the direct cause of rosette rather than infection of the soil with parasitic organisms from previously diseased trees.

Top soil and subsoil were taken separately in March, 1913, from the immediate vicinity of trees in the last stages of rosette at Belleview and at Tallahassee, Fla. This soil was shipped to Washington D. C., and in early June, 17 normal, recently germinated pecan seedlings were set in each of the four soil types. At the same time a like number of seedlings were set in the garden soil ordinarily used in the Department greenhouses. In both cases the top soil was a sandy loam. The Belleview subsoil was almost clear sand, while the Tallahassee subsoil was a pasty red clay. Observations were frequently made throughout two seasons, but no symptoms of rosette appeared in any case. Of course, a test of this kind with a small quantity of soil in a porous 8-inch pot must be rather inconclusive with reference to any effect of the chemical ingredients of the soil, but it was thought that if the rosettte were caused by any organisms living in the soil surely there would be a chance of at least some of the trees contracting the disease. Even from this point of view two seasons under observation are not sufficient, but taken in connection with the other rosette work the evidence at this stage of the experiment is perhaps worthy of record as tending to indicate the non-parasitic nature of the disease.

GERMINATION OF NUTS

In order to determine the communicability of the rosette, pecan nuts matured in the fall of 1912 on rosetted trees were planted in moist sand in one of the Department greenhouses in Washington, D. C. As they came up they were potted in garden soil and kept under observation during the spring, summer, and fall of 1913, and the summer of 1914. The nuts were obtained under the following conditions:

Of 12 nuts obtained directly from a rosetted branch, 9 germinated. Of 10 from a tree, most of which showed rosette, 9 germinated. Both lots were from the seedling orchard of Dr. W. P. Williams, Blackshear, Ga.

Of 93 nuts from a Frotscher pecan tree, showing rosette over the whole top, 52 germinated. This lot was sent by Mr. C. A. Reed, of the Bureau of Plant Industry, from the Parker orchard, Thomasville, Ga.

Of 25 nuts obtained from rosetted branches of Teche, Alley, Stuart, and Van Deman pecans from the orchard of Mr. W. P. Bullard at Albany, Ga., 18 germinated.

Of 4 nuts from a rosetted branch on an old seedling tree at Marion Farms, near Ocala, Fla., 3 germinated.

Of a second lot of nuts from Blackshear, Ga., number unknown, taken from a rosetted tree but not from a distinctly diseased branch, 28 germinated.

Out of all the nuts which germinated not a single seedling showed any symptoms of rosette, so that whatever the cause of the disease, it is apparently not transmissible through the seed.

ISOLATION OF MICROORGANISMS AND INOCULATIONS

To further test the communicability of the disease, several healthy nursery trees at Cairo, Ga., were inoculated with pieces of tissue from a badly diseased branch in August, 1902. The bark was removed from the latter, and bits of the wood scraped up with a sterile scalpel were placed in sterile water. Incisions were then made near the terminal buds of vigorous, healthy branches and bits of the diseased material inserted. This experiment was duplicated in August, 1906, when slices of diseased buds were inserted into the terminal branches of 11 nursery trees. The inoculated trees in both cases remained healthy.

During the fall of 1911 a series of attempts was made to isolate any organisms that might be present in various parts of diseased trees. Numerous Petri-dish cultures were made from the inner bark, cambium, wood, and pith of living rosetted twigs and from the pith and inner bark of the living roots ¼ inch to 3 inches in diameter. Pieces of the tissue in each case were transferred to beef agar and corn-meal agar. All these cultures remained sterile. Material for these and the following tests was obtained by the junior author from three orchards at Tallahassee, Fla., and from specimens received from Sacaton, Ariz.

With the partly dying tissues, however, many of the cultures gave bacteria and fungi. This was to be expected, since, as is well known, large numbers of saprophytic forms soon obtain entrance to tissues which have died from almost any cause. This is particularly true of tissues which have died from physiological causes, since they are not already infested with fungous or bacterial growth.

Out of 55 pieces of pith tissue from partly dying twigs, 39 remained sterile, 12 gave colonies of fungi, including an Aspergillus, a Penicillium, and a nonfruiting whitish fungus, and 4 gave as many different types of bacteria.

Out of 22 pieces of tissue taken at the juncture of dead and living wood on badly rosetted twigs, all developed fungous colonies.

Of 40 bits of inner bark taken from dying twigs, 27 were sterile, 6 developed fungous colonies, and 7 grew bacterial colonies.

Of 32 pieces of the inner bark taken from partly dying roots, 25 remained sterile, while 7 developed colonies of bacteria.

Since no constant form appeared in the cases where organisms did develop, it was thought highly improbable that the disease could be attributed to any of them, especially since a majority of the cultures remained sterile. Nevertheless, for the sake of completeness, greenhouse inoculations were made with all the different strains isolated, including 15 types of fungus and 17 types of bacteria. In each case needle-puncture inoculations were made in the tender growing tip and in the older bark of one or more pecan seedlings, and the latter were left under bell jars for several days. Check trees were similarly punctured with a sterile needle. Daily observations were made, and at the end of a week the needle punctures in the checks, and with two exceptions in all the inoculated trees, were healing over. The tips of these two inoculated trees were beginning to wither, but since their tissues were much broken up in the process of inoculation, this was thought to be due to mechanical injury. However, for further certainty several other trees were inoculated with these two bacterial strains, care being taken to injure the succulent tissues as little as possible. These all healed over without signs of infection.

Several examinations of healthy and rosetted roots showed a whitish, fungous weft on the young roots of healthy trees which was at first not found on those with rosette. It was thought possible that the pecan might be dependent on some mycorrhizal relation for its well-being and that the absence of the fungous symbiont gave rise to the diseased condition. However, diseased and healthy trees have been dug up in Texas, Georgia, Florida, and South Carolina, and in some cases the sterile, fungous weft has been found on healthy roots, sometimes on rosetted roots, but more frequently, so far as could be detected, it was absent. Moreover, of 300 healthy trees grown from seed in the Department greenhouses at Washington, D. C., those with and without fungous weft on the roots were about equal in number. Twelve isolations were made from specimens with this fungous growth, and all, when grown on corn-meal agar, developed typical Fusarium spores.

It is by no means demonstrated that rosette has no mycorrhizal relations, but the preponderance of evidence lies strongly on the positive side of the question. The apparent absence of fungi and bacteria from still living rosetted material, as shown by cultural tests, and the negative results of inoculations with organisms obtained from partly dead rosetted material strongly support the view that the disease is not of parasitic nature.

BUDDING AND GRAFTING EXPERIMENTS

NORMAL BUDS AND CIONS ON ROSETTED STOCKS

Four badly rosetted trees in the J. B. Wight orchard at Cairo, Ga., were budded from healthy Frotscher pecan trees in April, 1903. All lived and showed rosette the following season.

Eighteen rosetted trees in the orchard of Mr. G. W. Saxon, at Tallahassee, Fla., were cleft-grafted with normal cions from an old seedling tree in February, 1912. Most of the cions began to put out leaves in the spring, but all except three were destroyed by bud worms. These three put on a vigorous and apparently healthy growth the first part of the season, but toward fall and during the following summer symptoms of rosette were distinct in all three cases.

In an orchard of the Standard Pecan Co., at Monticello, Fla., two badly rosetted trees of each variety—Schley, Stuart, and Pabst—were budded with several buds from a healthy tree. Observations the following August showed two buds on each of the Schley pecans living, four and six buds on the two Stuart trees, and one bud on one of the Pabst trees. All shoots from these buds were badly rosetted.

ROSETTED BUDS AND CIONS ON NORMAL STOCK

In the spring of 1903, 24 buds subtended by distinctly rosetted leaves were put into healthy nursery trees, and 24 similar buds into healthy orchard trees of Mr. J. B. Wight, Cairo, Ga. Observations in midsummer, 1904, showed 13 living buds in the nursery, and of these 1 had a distinct case of rosette, 1 a trace, while the others were normal. Of the buds put into the orchard trees, 20 were living and only 1 had rosette. In the latter case the tree had developed rosette over the whole top subsequent to the budding operation. This being the only one behaving in this manner it can hardly be considered probable that the rosette in this case was transmitted through the bud.

One hundred buds from rosetted branches were worked on nursery seedlings at the same place in August, 1906. A large percentage lived, and in the following midsummer no traces of rosette could be found on any of them, though the trees from which the buds were taken still showed the disease.

In August, 1907, 82 more buds were inserted on healthy seedling stocks in the same nursery. Observations the following season (October, 1908) showed the same results as in the last experiment.

Twenty to thirty rosetted buds of each of the Schley, Pabst, and Stuart varieties were worked on nursery seedlings belonging to the Standard Pecan Co., Monticello, Fla., in August, 1912. In the following August, out of the 12 living Schley pecan buds 1 showed a doubtful trace of rosette; of the 10 living Pabst buds 1 showed a distinct and 1 a doubtful trace; and of the 10 living Stuart buds 1 showed a distinct

and 1 a doubtful trace of rosette. Counts in the two adjacent nursery rows on either side showed at least as high a percentage of rosette as those worked with diseased buds.

Buds from badly rosetted branches were taken from Tallahassee, Fla., and were worked on 8 healthy orchard trees at Glen St. Mary, Fla., in August, 1912. At the same time healthy buds were inserted in branches of 2 trees with distinct symptoms of rosette. Out of 30 to 35 diseased buds inserted, 14 developed, and the following August no rosette could be found on any of them. Of the 12 healthy buds on rosetted stock, 4 had lived. In the case of 1 bud the shoot was perfectly normal, but the tree as a whole had meanwhile recovered from rosette. The 3 others showed only traces of rosette, but the tree on which they were worked also had nearly recovered from the disease.

Grafting was also attempted in this connection at Washington, D. C. Sixty 1-year-old seedlings were grafted by the veneer method with rosetted cions from Cairo, Ga., but none of the cions developed.

In February, 1912, 105 badly rosetted cions from two orchards in Tallahassee were whip-grafted into a part of the general nursery of Mr. H. K. Miller, at Monticello, Fla. Nursery trees on all sides were grafted to healthy cions. The following August 45 cions were living. Of these, 7 showed traces and 2 had developed distinct symptoms of rosette. Counts in the adjacent general nursery showed about the same percentage of rosette.

It will be noted that normal buds and cions on rosetted stocks invariably gave rosetted shoots. Rosetted buds and cions on apparently healthy stocks, with but few exceptions, gave healthy shoots, and wherever exceptions occurred the percentage of rosetted shoots was no greater than in adjacent stocks worked with normal buds. The results here tend to show that pecan rosette is not caused by a perennial mycelium, or by bacteria, or by any infecting virus within the tissues of the host.

FERTILIZER EXPERIMENTS

A fertilizer test was started in March, 1902, in a badly rosetted orchard belonging to Mr. J. B. Wight, Cairo, Ga. Alternate rows were used for the five plots, and the intervening rows in each case were left untreated. Plot 1 received nitrate of soda; plot 2, lime; plot 3, cottonseed meal, acid phosphate, and kainit; plot 4, a liberal application of stable manure; plot 5, ground bone meal. Observations in the summer of 1904 in plot 1 showed 5 trees with the same amount of rosette as at the beginning of the experiment; 1 tree, better; and 5, worse. In plot 2 there was no change in 3 trees, but 10 others were worse. In plot 3, 7 trees were in the same condition as at the beginning, 1 was better, and 3 were worse. In plot 4, 9 trees were the same, 1 was better, and 3 were worse. In plot 5, 6 trees were the same, 2 were better, and 4 worse. Two check rows in the same orchard showed, respectively, 6 trees in the same condition, 1

better, and 6 worse; and 3 the same, 2 better, and 7 worse, showing that during the years in question a slight increase in the disease had taken place, and any effect of the fertilizers applied was scarcely discernible. The plot treated with lime stands out somewhat from the rest, since there was distinctly more increase in the disease here and no tree showed signs of recovery.

In April, 1911, a 16-plot fertilizer test was started among nonrosetted 8-year-old Georgia Giant and Nelson pecans in an orchard at Baconton, Ga., at that time the property of Mr. Chas. M. Barnwell. The soil is a sandy loam underlain at 1½ to 2 feet by red clay. Two rows of each variety were taken through the block, giving 4 trees of each variety to a plot. The same general scheme of fertilizer combinations was used as that employed by Mr. M. B. Waite in his apple-nutrition experiments, and annual applications were made, except in the case of lime, which was used only at the beginning of the experiment. The land was cropped to winter oats, followed by cowpeas, the first two seasons, and the third season velvet beans only were grown in the centers. At the last observation there was but little apparent difference among the fertilized trees in general vigor and length of growth, color of foliage, and quantity of nuts, but the two check plots bore foliage of a conspicuously paler color. The condition of the various plots in August, 1913, is shown in Table I.

TABLE I.—*Summary of results from a fertilizer test with pecan trees at Baconton, Ga.*

Plot No.	Fertilizer.[a]	Number of trees with rosette.	Number of doubtful cases.	Number of healthy trees.	Average increase in diameter.
					Inches.
1	Lime	1	.....	7	1.23
2	Lime and nitrate af soda	3	2	3	.99
3	Lime and cotton-seed meal	.....	.....	8	1.25
4	Lime and muriate of potash	1	.....	6	.97
5	Lime and sulphate of potash	.....	2	6	1.29
6	Lime and acid phosphate	1	.....	6	1.27
7	Lime and Thomas phosphate	.....	.....	7	1.01
8	Lime, muriate of potash, and nitrate of soda	2	3	3	1.12
9	Lime, muriate of potash, and acid phosphate	5	.....	3	1.00
10	Lime, acid phosphate, and nitrate of soda	6	1	.....	1.33
11	Lime, muriate of potash, nitrate of soda, and acid phosphate	2	.....	6	.87
12	Muriate of potash, nitrate of soda, and acid phosphate; no lime	.....	2	4	1.48
13	Muriate of potash and acid phosphate; no lime	.....	.....	8	1.08
14	Stable manure; no lime	.....	.....	8	.94
15	Stable manure and ground bone; no lime	.....	.....	8	1.05
16	Control; no lime	.....	.....	7	1.03

[a] Fertilizers were applied at the following rates: Lime (CaO, acted on jointly by air and water), 1 bushel per tree; nitrate of soda, 8 pounds; cottonseed meal, 32 pounds; muriate and sulphate of potash, 8 pounds; acid phosphate, Thomas phosphate, and ground bone, 24 pounds; stable manure, a liberal application.

It will be noted from Table I that rosette occurred on all but two of the plots receiving an application of lime. In the five unlimed plots there were only two doubtful cases of rosette and these two trees directly bordered a limed plot. Care was taken in spreading the fertilizers on the side bordering a contiguous plot not to distribute to the middle, but the lime was spread to the dividing line, and, hence, there was a chance of its affecting the adjoining unlimed plot. The largest number of cases and those showing the most advanced stages occurred on the two limed plots, Nos. 9 and 10, treated, respectively, with acid phosphate and muriate of potash and with acid phosphate and nitrate of soda. Thus, while the results of this test have so far been partly negative, they have at least tended to show that some relation exists between pecan rosette and the constituents of the soil.

A fertilizer test was started in April, 1912, in the badly rosetted part of a 7-year-old orchard belonging to Mr. G. W. Saxon, at Tallahassee, Fla. The soil here is a sandy loam underlain by a stiff red clay. Thinking that possibly the lack of proper drainage might be a factor predisposing to rosette, the subsoil around six badly diseased trees was dynamited. Three 6-foot holes were bored at 10 to 12 feet from the trees and ¼ to ½ pound of dynamite was used to each hole. Applications of sulphur flour and of copper sulphate to the soil were also tried, each at the rate of 6 pounds to the tree. With the exception of lime, which was applied at the rate of one-third of a bushel to the tree, the fertilizers were used at the rate noted for the preceding experiment. The results of this experiment are given in Table II.

TABLE II.—*Summary of results from a fertilizer test with pecan trees at Tallahassee, Fla.*

Plot No.	Fertilizer.	August, 1912.		August, 1913.		
		Number of trees with rosette.	Number of normal trees.	Number of trees with rosette.	Number of doubtful cases.[a]	Number of normal trees.
1	Lime, muriate of potash, nitrate of soda, and acid phosphate	9	1	9	[b] 1	.....
2	Muriate of potash	3	.....	3	.....	.....
3	Acid phosphate	4	.....	3	[b] 1	.....
4	Control, subsoil dynamited	6	.....	6	.....	.....
5	Control, untreated	7	.....	6	[b] 1	.....
6	Nitrate of soda	5	.....	3	[b] 2	.....
7	Muriate of potash	5	.....	4	[b] 1	.....
8	Acid phosphate and nitrate of soda	3	.....	3	.....	.....
9	Muriate of potash, nitrate of soda, acid phosphate	6	.....	6	.....	.....
10	Stable manure	1	3	2	1	1
11	Thomas phosphate	2	1	2	.....	1
12	Lime	.....	2	1	.....	1
13	Cottonseed meal	2	1	3	.....	.....
14	Copper sulphate	1	1	1	.....	1
15	Sulphur flour	1	1	1	.....	1

[a] As these trees were badly affected with rosette at the beginning of the experiments, it is probable that their death was at least in large measure due to this disease.
[b] Dead.

As will be observed from the data given, the results were largely negative. There were no signs of recovery, and in the case of stable manure (plot 10), lime (plot 12), and cottonseed meal (plot 13), there was an increase in the number of rosetted trees. It should be noted that most of the trees having rosette at the start were well-advanced cases.

At the same time, the subsoil was dynamited around three 15-year-old rosetted trees in the orchard of Mr. G. G. Gibbs. The results in this case were likewise negative as shown by observations after one and two years. The results of dynamiting the subsoil in these two orchards, together with observations showing the absence of pecan rosette in swampy land, seem to indicate that the disease is not due directly to lack of proper subsoil drainage.

The soil around two rosetted trees in the J. B. Wight orchard, Cairo, Ga., in the spring of 1907, was treated with copper sulphate and magnesium sulphate at the rate of 1 pound of each for every inch in diameter of the trunk. The trees were decidedly injured by this treatment, and the diseased condition continued as before. A negative result followed the use of 8 pounds of copper sulphate around a single 9-year-old rosetted tree at Baconton, Ga.

The soil around each of 22 rosetted trees in the 4-year-old Davenport orchard at Belleview, Fla., was treated in June, 1912, with 2 to 4 pounds of copper sulphate, according to the size of the tree. By the following midsummer 7 trees had recovered, 7 were somewhat improved in appearance, and 8 were either in the same or in a worse condition than at the beginning. In the same 40-acre block, 117 out of 389 rosetted trees had recovered in the same period. In other words 31 per cent of the trees treated with copper sulphate recovered and also 30 per cent of the untreated trees in the same block. A considerable number of the untreated trees were also improved in appearance. Both groups of trees were fertilized by the owner with a complete commercial fertilizer at the rate of 8 pounds to the tree.

The copper-sulphate treatment has from time to time been recommended by a number of orchardists, and in a few cases observed by the junior author some apparently beneficial results have occurred. But the usual failure of the grower to run proper checks with an experiment, together with the fluctuation of the disease without any treatment, lends a rather doubtful character to the results. At any rate, this treatment is not to be recommended, except in an experimental way, until further tested out.

SPRAYING EXPERIMENT

Five rosetted trees in the J. B. Wight orchard at Cairo, Ga., were given three applications of Bordeaux mixture—in March, April, and May, 1903. No positive results from this experiment were discernible.

ORCHARD RECORDS

Rosette records for periods varying from 2 to 12 years have been kept in several orchards in Georgia, Florida, and South Carolina.

Of 159 trees observed in the J. B. Wight orchard for three successive seasons (1902 to 1904), 24 were healthy for all three seasons, 17 were healthy at the beginning, but later contracted the disease, 13 had rosette, but recovered, 108 were rosetted throughout the period, and 7 fluctuated each season.

Eighty trees in the same orchard were observed in 1902, 1903, 1904, 1912, and 1913. So far as these observations go, 30 trees remained normal throughout the period, 7 were normal, but contracted the rosette, 12 were rosetted, but recovered, 8 were rosetted throughout, and 21 fluctuated back and forth between the normal and rosetted condition.

Observations on 274 trees in the same orchard during 1912 showed 136 trees with the rosette, and during the following season 35 trees more had contracted the disease. The cultivation and fertilization had not been varied.

In a 40-acre block of the Davenport orchard at Belleview, Fla., containing 1,069 trees, 389 had the rosette in 1912 and 256 in 1913. All had received the same treatment, except for the few trees used in the copper-sulphate test, and as previously noted this treatment gave negative results. Of the three varieties present, 19 per cent of the Van Deman, 25 per cent of the Stuart, and 28 per cent of the Teche pecans had rosette in 1912.

Sixty-two soil borings were made to 6 and 9 feet from the surface and in the vicinity of both healthy and rosetted trees. Of 31 trees in a clay to sandy-clay subsoil, 13 had the rosette and 18 were normal. Of 23 trees in a sandy subsoil, 9 were rosetted and 14 were normal. Of 8 trees in a subsoil containing considerable quantities of a soft lime rock, all were healthy. In the first two groups the difference in the number of diseased and healthy trees was not conspicuous. On account of the entire absence of rosette in the third group, partial analyses of the subsoil around three trees were made. In sample No. 1 the percentages of lime, magnesium, and phosphorus, computed as the oxids, were 9.68, 0.82, and 8.32, respectively; in sample No. 2, 5.42, 1.09, and 6.03; and in sample No. 3, 0.58, 0.99, and 3.44. More or less clay was present in all the samples, and in No. 2 there was a considerable admixture of creolin.

In a small orchard of 96 trees belonging to Mr. G. G. Gibbs, at Tallahassee, Fla., 38 trees were rosetted in 1912, and during the following season 4 of these recovered. In another orchard of 116 trees on the same farm, with similar topography and apparently similar soil, 6 were rosetted in 1912 and 12 the following season.

In the G. W. Saxon orchard of 231 trees at Tallahassee, Fla., 125 trees had rosette in 1912 and 173 in 1913. Most of the orchard had received

no fertilizer for several seasons, and where fertilizer had been applied no distinct difference in the rosette could be detected.

In the W. P. Bullard orchard near Albany, Ga., 291 out of 646 trees were rosetted in 1912. By the following season 101 more trees had contracted the disease. Fertilization and cultivation were uniform for the two seasons.

In a block of 233 trees belonging to Mr. H. K. Miller, at Monticello, Fla., 81 trees had the rosette during 1912 and 1913. Fertilization was uniform for both years, and the soil was a sandy loam underlain by a rather stiff, red, sandy clay.

Observations were taken during 1912 and 1913 in three blocks belonging to the Standard Pecan Co., at Monticello, Fla. Out of 406 trees observed in the first block 231 had rosette in 1912 and 254 in 1913. In the second block of 450 trees 119 were rosetted at the first observation and 121 at the second. In the third block of 570 trees, 42 were rosetted in 1912, and 34 in 1913. A part of all three blocks were in swamp land that had not been fully drained, and the absence of rosette here was conspicuous. The soil in the remainder of the three blocks was a sandy loam underlain by a red clay or sandy clay. For three seasons preceding 1912 a complete commercial fertilizer was used. In 1912 a small application of stable manure was made around each tree.

In the small orchard of 100 trees belonging to Dr. R. B. Garnett, at St. Augustine, Fla., 3 were rosetted, both in 1912 and 1913, 7 were rosetted but recovered during the second year, and 6 new cases developed during the second year. The soil is a sandy loam underlain by a clear-sand subsoil, with the water table 3 to 3½ feet below the surface. Fertilization was uniform over the orchard, except that the soil around the 10 trees showing rosette in 1912 was treated with lime and copper sulphate. Seven of these trees recovered, but no checks were run to verify the result.

In a block of 371 trees belonging to Mr. M. O. Dantzler, at Orangeburg, S. C., 146 trees had rosette in 1912 and 137 in 1913. Fertilization and cultivation were uniform during the two seasons.

It is evident from these records that rosette fluctuates from year to year without any variation in the treatment given by the grower and that diseased trees may apparently make a complete recovery and remain healthy for an indefinite period, or after a season or two they may again contract the disease. It should be stated here that in the majority of cases the trees recovering from rosette had not reached the staghorn stage. However, a considerable number of trees with terminals dying back from the disease have been seen to recover and remain normal through the one or more seasons they have been subsequently under observation by the authors. From the variations in rosette recorded from year to year under uniform cultivation and fertilization it seems

highly probable that seasonal climatic changes, such as variation in the water content of the soil, may have at least an indirect relation to the prevalence of the disease.

ASH ANALYSES [1]

Complete ash analyses were made of rosetted and healthy leaves and of rosetted and healthy twigs from Cairo and Dewitt, Ga., and from Belleview, Fla. In each case the diseased and healthy material was from trees of the same age and variety and had received similar cultivation and fertilization in the same orchard. The pure-ash analyses are given in Tables III to V.

TABLE III.—*Ash analyses of leaves and twigs of the Stuart pecan from Cairo, Ga., September, 1912*

Constituent.	Leaves.		Twigs.	
	Rosetted.	Normal.	Rosetted.	Normal.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Total percentage of pure ash	5.59	5.02	5.47	5.49
SO_3	3.60	3.02	3.69	3.11
P_2O_5	15.47	21.11	12.26	16.59
Cl	.33	.17	.26	.14
K_2O	23.36	18.35	23.01	16.01
Na_2O	.98	.83	1.18	1.02
CaO	31.48	34.09	34.14	48.19
MgO	21.50	14.09	24.45	14.78
SiO_2	3.25	8.33	.96	1.24
Total	100.04	100.09	100.04	101.18

TABLE IV.—*Ash analyses of leaves and twigs of the Van Deman pecan from Belleview, Fla., September, 1913*

Constituent.	Leaves.		Stems.	
	Rosetted.	Normal.	Rosetted.	Normal.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Total percentage of pure ash	4.68	4.61	3.68	3.37
SO_3	7.31	5.68	6.19	4.69
P_2O_5	7.57	8.86	8.24	12.24
Cl	.38	.35	.30	.40
K_2O	18.76	16.03	21.35	25.61
Na_2O	.38	.34	2.23	3.24
CaO	37.75	32.45	44.16	36.56
MgO	20.66	20.30	17.40	16.71
Fl_2O_3, Al_2O_3	1.90	.65	Trace.	Trace.
Mn_3O_4	.40	.58	.23	.26
SiO_2	4.83	14.30	.26	.29
Total	99.94	.....	98.36	.....

[1] The ash analyses here given were made by the Bureau of Chemistry, Department of Agriculture.

TABLE V.—*Ash analyses of leaves and twigs of the Schley pecan from Putney, Ga., September, 1913*

Constituent.	Leaves.		Stems.	
	Rosetted.	Normal.	Rosetted.	Normal.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Total percentage of pure ash	4.72	4.78	2.16	2.18
SO_3	6.76	5.83	6.49	5.11
P_2O_5	6.23	6.52	6.40	7.28
Cl	.69	.26	.38	.32
K_2O	23.03	14.12	22.37	19.03
Na_2O	.49	.34	2.82	2.51
CaO	35.12	42.04	40.04	42.27
MgO	16.74	16.42	16.95	19.23
Fe_2O_3, Al_2O_3	3.03	4.84	1.43	2.23
Mn_3O_4	1.19	2.44	.61	1.09
SiO_2	6.45	7.23	1.76	.74
Total	104.45	104.82	101.41	101.99

Most of the differences between the pure ash of healthy and of rosetted material were not constant in the three sets analyzed. In both leaves and twigs from Cairo, Ga., the magnesium content was much higher in the diseased material, but in the two other sets the percentage was nearly the same in both diseased and healthy material.

The percentage of phosphorus was greater in the normal leaves of two sets and in the normal twigs of one set. In the other cases the percentage was about the same in both healthy and diseased material.

The calcium content was greater in the normal leaves and twigs of two sets and considerably less in the remaining set of material.

The percentage of potassium was greater in all the rosetted material, with the exception of one set of rosetted and normal twigs, where there was slightly more potassium in the normal.

Other differences shown by the analyses are either slight or greatly variable.

DISCUSSION OF RESULTS

PARASITISM VERSUS NONPARASITISM

The following experimental data have a bearing on the question of possible parasitism or upon nonparasitism.

Out of 144 nuts collected in different localities from badly rosetted branches, 91 germinated and none of the seedlings gave symptoms of the disease during the two seasons under observation. These nuts were placed in the greenhouses of the Department of Agriculture at Washington, D. C., which is far removed, both as to locality and environment, from the orchards where the nuts were obtained, but the conclusion from

this experiment nevertheless seems justifiable that whatever the cause of the disease it is not transmissible through the seed (see p. 155).

Inoculation of tender growing tips with bits of diseased tissue gave negative results. Attempts at isolation of micro-organisms from living rosetted material gave negative results in all cases. Various fungi and bacteria were isolated from partially dead material obtained in four different orchards, but no constant form appeared and inoculations gave negative results (see p. 156). These results strongly support the view that pecan rosette is not of a parasitic nature.

Mycorrhiza have been found promiscuously on both normal and rosetted trees, or perhaps more often the roots of both normal and rosetted trees have appeared to be free from any such fungous growth. It is probable that the disease is not due to the presence or absence of mycorrhiza (see p. 157).

Where normal buds or cions were worked upon rosetted stocks they have invariably developed the disease during the same or the following season, except in a few cases where the trees used as stock had in the meantime themselves recovered. With few exceptions rosetted buds and cions worked upon apparently normal nursery and orchard trees developed into normal shoots. Where rosetted shoots developed their percentage was no greater than in contiguous nursery rows or orchard trees worked to normal buds or cions (see p. 158). The results here tend to show that rosette is not caused by a perennial fungus mycelium, by bacteria, or by any infecting virus within the tissues of the pecan.

Healthy nursery trees set in large pots of top soil and of subsoil taken around badly rosetted trees remained normal during the two seasons under observation (see p. 155). This series of tests was carried out in the Department greenhouses at Washington, D. C., small quantities of the soil being used under entirely different environment than present in the location from which obtained. The results are therefore not taken as conclusive, but merely as tending to indicate the nonparasitic nature of the disease.

Transplanting of healthy nursery trees to holes from which rosetted trees had been removed gave a very high percentage of rosette in the replants, while transplanting badly rosetted trees to situations where less rosette or none at all had been observed gave a very high percentage of recovery (see p. 152). By comparison of the results of these two tests it appears that a soil relation is the important factor in causing rosette rather than any transmission of the disease from one tree to another.

Thus, from the nontransmission by seed, the negative results of isolation and inoculation tests, the varying presence and nonpresence of mycorrhiza, the budding and grafting tests, and the transplanting work, the nonparasitism of pecan rosette is considered a reasonable assumption.

RELATION TO THE SOIL

The relation of pecan rosette to the soil is partially elucidated by the following experimental data.

Severe pruning of rosetted trees or cutting back to a stump gave negative results (see p. 152). The new growth was often healthy in appearance, but toward autumn or during the following season symptoms of rosette invariably reappeared (Pl. XXVIII, fig. 2). The fact that in no case was the disease eliminated by this treatment is at least not unfavorable to the view that it is contracted directly or indirectly through the soil.

Transplanting of healthy nursery trees to holes from which rosetted trees had been removed gave a very high percentage of rosette in the replants (see p. 152). These results point toward the conclusion that some relation exists between rosette and the soil, either directly through the soil itself or through previous infection of the soil by rosetted trees.

The transplanting of badly rosetted trees to situations where less rosette or none at all has been observed gave a very high percentage of recovery (see p. 153). All rosetted trees replanted at Washington, D. C., recovered, though many of them were dying back with the disease when first taken up (see p. 154). In the latter cases the entire change of both soil and climate is held accountable for the uniform recovery of all rosetted trees. All these transplanting tests with rosetted trees tend to indicate the soil relation as the direct cause rather than infection of the soil with a parasitic organism or virus from previously diseased trees.

The results of the fertilizer experiments were partially negative (see p. 159). However, in a 16-plot test upon normal trees at Baconton, Ga., 9 out of 11 limed plots developed cases of rosette, while 1 out of 5 unlimed plots showed doubtful traces on two trees bordering a limed plot. Careful observation at the beginning of the experiment did not reveal a single case of rosette, and at the last observation no rosette was found in contiguous parts of the orchard. The plots receiving lime, acid phosphate, and nitrate of soda and those receiving lime, acid phosphate, and muriate of potash developed by far the most rosette. In fertilizer experiments at two other points considerably more rosette developed on limed than on unlimed plots. These results, while not in all cases very definite, have at least tended to show that some relation exists between rosette and the constituents of the soil and that the lime content alone or in combination with other substances may have a varying causative effect.

Dynamiting the subsoil in two different orchards gave negative results (see pp. 161–162). This fact, together with observations showing the absence of rosette in swampy land (see p. 150), seems to indicate that the disease is not directly due to lack of proper subsoil drainage.

Orchard records of individual trees over periods of 2 to 12 years show considerable fluctuations in the disease, irrespective of treatment and without any special treatment (see p. 163). Trees with a mild or moderate attack frequently recovered, and even when the staghorn stage

had been reached, trees occasionally regained their normal appearance without any artificial treatment. From the seasonal variations in rosette recorded from year to year under conditions of uniform cultivation and fertilization it seems highly probable that seasonal climatic changes, such as variation in precipitation, may have at least an indirect relation to the prevalence of the disease. In large orchards the more or less simultaneous appearance of rosette in patches, together with its usual limitation to these areas, clearly suggests some definite connection with the soil conditions.

In general, the ash analyses of rosetted and healthy material showed slight to highly variable differences, so that very little positive light was thrown upon the problem by this part of the work (see p. 165). In both leaves and twigs from one orchard the magnesium content was much higher in the rosetted than in the normal material, but in two other sets of analyses the percentage was nearly the same in both diseased and healthy material. The percentage of phosphorus was greater in the normal leaves of two sets and in the normal twigs of one set. In the other cases the percentage was about the same in both healthy and diseased material. The calcium content was greater in the normal leaves and twigs of two sets and considerably less in the remaining set. The percentage of potassium was greater in all the rosetted material, with the exception of one set of rosetted and normal twigs, where there was slightly more potassium in the normal. Other differences shown by the analyses are either slight or greatly variable.

It thus appears from the results of experiments in pruning and cutting back, transplanting tests, fertilizer experiments, results of subsoil dynamiting, and orchard records that the disease is directly or indirectly caused by some soil relation. On account of their variable character, the ash analyses shed little light on the problem.

COMPARISONS WITH OTHER DISEASES

Leaf-hopper injury suggested itself at one time as a possible cause of rosette of pecans. The rather far-reaching effects of the work of this insect were known, and the demonstration of their causal relation to curly-top of beets [1] lent further plausibility to this theory. However, extensive observations have failed to disclose any connection between this insect injury and rosette. Leaf-hopper injury has occasionally been seen on pecans, but its symptoms are distinct and it has occurred both in the presence and absence of rosette. The leaves are often yellowed around the margin, somewhat curled, and if attacked while young, their growth is considerably interfered with. But there are not the distinct yellow mottling over the whole blade between the veins, the raised appearance of the latter, and the tendency to reduction of the leaf blade, followed by the dying back of the shoot from the tip.

[1] Shaw, Harry B. The curly-top of beets. U. S. Dept. of Agr., Bur. Plant Indus., Bul. 181, 46 p., 9 fig., 9 pl. 1910.

Neither is rosette to be confused with sun scald, or "winterkill," which affects young trees especially and manifests itself in the death of the cambium at the base of the trunk. The roots are usually uninjured and healthy sprouts soon put out from the base. Again, frost injury may kill back the tips in a manner somewhat analagous to the staghorn stage of rosette, but here the foliage symptoms are lacking, and in rosette the death of the branches is usually most prominent in late summer, a season far removed from frost.

Both the yellows and rosette of peach in a general way suggest pecan rosette. However, though some symptoms may be common to all three diseases, the complete clinical picture is distinct in each case. Some of the most prominent symptoms of peach yellows consist in the production of abnormally long, spindling branches in dense groups due to the putting out of normally dormant and possibly adventitious buds. Yellowing of the leaves always occurs at some time during the course of the disease; but in spite of the name "yellows," the leaves are often abnormally dark green in a case of recent attack. The pushing into growth of normally dormant buds and also possibly of adventitious buds is likewise characteristic of pecan rosette, but the axes are shortened rather than elongated as in peach yellows. In this shortening of the axes of growth the resemblance to peach rosette is seen. In the last-named disease the twigs are so much shortened that the leaves as they develop become clustered into a compact rosette. Peach trees affected with rosette usually die during the first and never survive the second season, and those suffering from yellows rarely survive more than five seasons, but rosetted pecan trees have been known to live for 15 years. Moreover, no case is on record of a recovery from peach yellows or peach rosette, while recovery from a moderate attack of pecan rosette is frequent, and not rare, even for the later stages. Furthermore, from the experiments outlined in this paper it appears that pecan rosette is not transmissible either through the seed or by budding or grafting, while the infectious nature of peach yellows and rosette is well established through experimental bud transmission to healthy trees.

A striking resemblance is to be observed between pecan rosette and ordinary chlorosis of various trees. In moderate cases of the latter disease yellowing of the leaves occurs without notable change in form or size, and the conspicuous dying back of the branches from the tip is lacking, but all gradations occur between such cases and those where the symptoms closely simulate rosette of pecans.

The spike disease of pineapples[1] bears some general resemblance to rosette of pecans both as to effect and apparent cause. The leaves of affected pineapples become contracted at the base so as to form a spike-like blade, and the color of the leaf also becomes modified. It is claimed that cottonseed meal, sulphate of ammonia, kainit, muriate of potash,

[1] Rolfs, P. H. Pineapple fertilizers. Fla. Agr. Exp. Sta. Bul. 50, p. 97–99. 1899.

and acid phosphate aggravate the disease, while nitrate of soda and sulphate of potash seem to have a beneficial effect upon diseased plants. The fertilizer tests with pecans point toward the conclusion that rosette is also a nutrition trouble.

PROBABLE NATURE OF PECAN ROSETTE

Rosette of pecans evidently belongs among the chlorotic diseases of plants grouped by Sorauer [1] into two main classes: (1) Noninheritable and noninfectious diseases due mostly to improper nutritive supply or to injurious physical conditions, and (2) inheritable and infectious diseases due probably to enzymatic disturbances. From the results of the experiments and observations outlined in this paper it seems legitimate to conclude that pecan rosette should be placed in the first class of chlorotic diseases—viz, those nontransmissible diseases caused by improper nutritive supply or injurious physical conditions.

From the definite sequence of a considerable number of symptoms in pecan rosette it would seem more probable that the disease is directly caused by one set of conditions which, however, may be indirectly influenced by other conditions such as amount of rainfall, etc., rather than that this same set of symptoms may in different localities be caused by entirely different sets of conditions. Such a statement can not be laid down as a demonstrated fact, but the general knowledge of both plant and animal pathology renders it extremely probable.

The spike disease of pineapple, to which reference has previously been made, has been rather clearly demonstrated to be caused by improper nutritive supply, and it seems rather probable that pecan rosette is of a similar nature. From its wide distribution pecan rosette is clearly not confined to any one general soil type, but it is entirely possible that in those soils subject to the disease the proper balance between two or more soil ingredients may not be maintained. For example, the effects of a lack of proper balance between lime and magnesium are fairly well known, and it is possible that some such condition as this may be responsible for rosette. Indeed, the results of our preliminary fertilizer experiments point in this direction. The lime used was a high-grade stone lime purchased in barrels, but its content of magnesium was not known. The percentage of rosette was distinctly higher in the plots treated with lime, but in the absence of an exact analysis of the lime used it can not be determined from these tests whether the injury came from the lime alone or whether magnesium played a part in causing the disease. However, the chemical analysis of subsoil from the Davenport orchard, at Belleview, Fla. (see p. 163), would seem to show that lime of itself is not injurious. In this orchard the only type of subsoil entirely free from pecan rosette gave by analysis 0.58 to 9.68 per cent of lime, 3.44 to 8.32 per cent of phosphorus, and 0.82 to 1.09 per cent of magnesium, all com-

[1] Sorauer, Paul. Handbuch der Pflanzenkrankheiten. Aufl. 3, Bd. 1, p. 308. Berlin, [1906].

puted as oxids. Lime was present as both carbonate and phosphate. It will be noted that the magnesium content was low. Considerable clay was present in all these cases and frequently with a considerable admixture of creolin.

The possibility of some relation to soil organisms is not entirely precluded, but it is thought that the direct cause will ultimately be found in some lack of balance in the nutritive supply, or possibly in some toxic organic substance or substances in the soil. The large group of physiological diseases to which pecan rosette seems to belong constitutes one of the most baffling series of problems now before the pathologist; and although a large number of workers have been investigating these diseases, none of them has as yet been fully worked out, either as to cause or control.

CONTROL

No great or constant difference in varietal resistance has been observed among the common orchard varieties. In one orchard a certain variety may have a much higher percentage of rosette than some other variety, but in another place the relative amount on the same two varieties is just as likely to be reversed. This has been shown clearly by orchard records in widely separated localities. Evidently the difference in apparent resistance in such cases is due either to a differenc in soil conditions in the two parts of the orchard or to a difference in the resistance of the stocks to the inciting cause. That there is sometimes a difference in the true resistance of the stocks seems evident from the fact that of two trees of the same variety growing side by side (1 foot to several rods apart) one may have rosette and the other appear perfectly normal. If the cause of the disease lies in the soil, as appears to be the case, such an influence of the stock would naturally be expected. There appears to be little doubt then as to the existence of a difference in the resisting power toward rosette, but orchard records and observations tend to show that this difference is usually manifested through the stock rather than through the variety worked upon it.

It should be added that until more is definitely known as to the direct cause of pecan rosette little can be said of its control by use of resistant stocks or by other methods. Good care and fertilization are to be recommended, but until more is known of the lime-magnesium balance in relation to rosette, orchardists should test the effects of lime upon a few trees before using it on a commercial scale. The use of copper sulphate upon the soil, though favored by many growers, can hardly be recommended as a remedy for rosette without more conclusive data as to its efficacy than have yet been forthcoming (see p. 162). The results of our experiments have shown that pruning as a remedial measure is of no avail. Since there is no evidence that pecan rosette is transmissible from tree to tree, the cutting out of orchard trees showing only traces of the disease is hardly to be recommended, because they

very often completely recover. However, with well-advanced cases, especially where dying back of the branches is prominent, there is so little chance for recovery that it would seem better to replant with sound, healthy, new trees, notwithstanding the fact that the unfavorable soil conditions may persist and cause a second failure.

As to the advisability of using rosetted nursery stock, no absolute statements can be made with the present state of knowledge concerning the cause of the disease and varying resistance of the stock to that cause. However, orchard and nursery records show rather clearly that a difference in resistance of stock does exist. This being granted, it is reasonable to suppose that among nonrosetted and rosetted stocks in the same nursery the latter, if ultimately set out in soils tending to cause rosette, will be far more likely to give rosetted orchard trees than the former. This theory is borne out by the fact that in one large orchard where records of the condition of the nursery stock used in planting were kept, observations after several years showed a much higher percentage of rosetted trees from the rosetted stock than from the nonrosetted stock. It is true that the rosetted stocks were set together in one part of the orchard and that some difference in soil constituents not revealed by soil borings may have caused the difference in prevalence of the disease, but this seems hardly probable.

Hitherto, pecan nurserymen have paid little attention to the presence of rosette in the nursery stock used in budding and grafting except in extreme cases of the disease, and it is thought by the authors entirely possible that part of the wide prevalence of the disease may be due to the dissemination of susceptible stock from the nurseries. The disease is a serious one on account of the fact that crop production and recovery from well-advanced stages are seldom seen, and for the good of the pecan industry in general the number of cases of rosette should be kept at a minimum. In view of these facts, the discarding of all rosetted nursery stock is to be strongly recommended.

SUMMARY

Pecan rosette has been rather generally recognized by growers as a serious disease almost from the inception of pecan orcharding. It does not appear to be limited to any particular soil type, topography, or season. The disease first makes itself evident through the putting out of undersized, more or less crinkled, and yellow-mottled leaves. The veins tend to stand out prominently, giving a roughened appearance to the leaf blade, and the lighter areas between the veins are usually not fully developed. The axes of growth are usually shortened, so that the leaves are clustered together into a sort of rosette. In well-marked cases the branches usually die back from the tip, and other shoots are developed from normal or adventitious buds, only in their turn to pass through the same series of symptoms.

The nonparasitism of the disease seems rather definitely established experimentally from the nontransmission by seed, the negative results

of isolation cultures and inoculation tests, the varying presence and non-presence of mycorrhiza on both healthy and rosetted trees, the budding and grafting tests, and the transplanting experiments.

It appears from the results of experiments in pruning and cutting back, transplanting tests, fertilizer experiments, results of subsoil dynamiting, and orchard records that the disease is directly or indirectly caused by some soil relation. On account of their variable character the ash analyses have shed but little light on the problem.

Leaf-hopper injury has been observed on pecans, but is distinct from rosette and has occurred both in the presence and the absence of the latter disease. Sun scald, or "winterkill," manifests itself in the death of the cambium at the base of the trunk and is not likely to be confused with rosette. Frost injury may simulate rosette in the killing back of the terminals, but the other rosette symptoms are lacking. Rosette and yellows of peach in a general way suggest pecan rosette, but though some symptoms may be common to all three diseases the complete clinical picture is distinct in each case. A striking resemblance is to be observed between pecan rosette and ordinary chlorosis of various trees, where all gradations occur from mere yellowing of the leaves to cases where the symptoms closely simulate rosette of pecans. The spike disease of pine-apples also bears some general resemblance to the rosette of pecans, both as to effect and apparent cause.

Observations and experimental evidence point to the conclusion that pecan rosette belongs among the chlorotic diseases of plants grouped by Sorauer into two main classes: (1) Noninheritable and noninfectious diseases, due mostly to improper nutritive supply or to injurious physical conditions, and (2) inheritable and infectious diseases, due probably to enzymatic disturbances. It seems legitimate to conclude from the data outlined in this paper that pecan rosette belongs in the first group. The evidence strongly points in the direction that the disease is caused by improper nutritive supply, and it seems probable that it is directly related to a lack of balance between two or more soil ingredients. The possibility of some relation to soil organisms is not entirely precluded, but it is thought that the direct cause will ultimately be found in some lack of balance in the nutritive supply, or possibly in some toxic organic substances in the soil.

There appears to be little doubt as to a difference in resisting power toward rosette, but orchard records and observations tend to show that this difference is usually manifested through the stock rather than through the variety worked upon it. Good care and fertilization are to be recommended, but the effects of lime should be tested upon a few trees before using it on a commercial scale. Pruning is of no avail as a remedial measure. Trees showing only traces of rosette may be left in the orchard, but all advanced cases should be cut out and replanted. On account of resistance versus susceptibility of stock, the discarding of all rosetted nursery trees is to be strongly advised.

PLATE XXIV

Fig. 1.—One normal pecan leaf and two leaves with rosette from Dewitt, Ga. Note the partial reduction of the leaf blade to the midrib.

Fig. 2.—Pecan shoot with early symptoms of rosette.

1

2

PLATE XXV

Rosetted pecan leaf showing perforations due to the failure of part of the mesophyll to develop. About natural size. From Cairo, Ga., October, 1903.

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PLATE XXVI

Fig. 1.—Pecan shoot in advanced stages of rosette. Note the raised veins, perforations, and reductions in area of leaf blade; also dying back of terminals. From Cairo, Ga., 1903.

Fig. 2.—Normal pecan shoot for comparison with rosetted shoot. From Cairo, Ga., July, 1903.

2

1

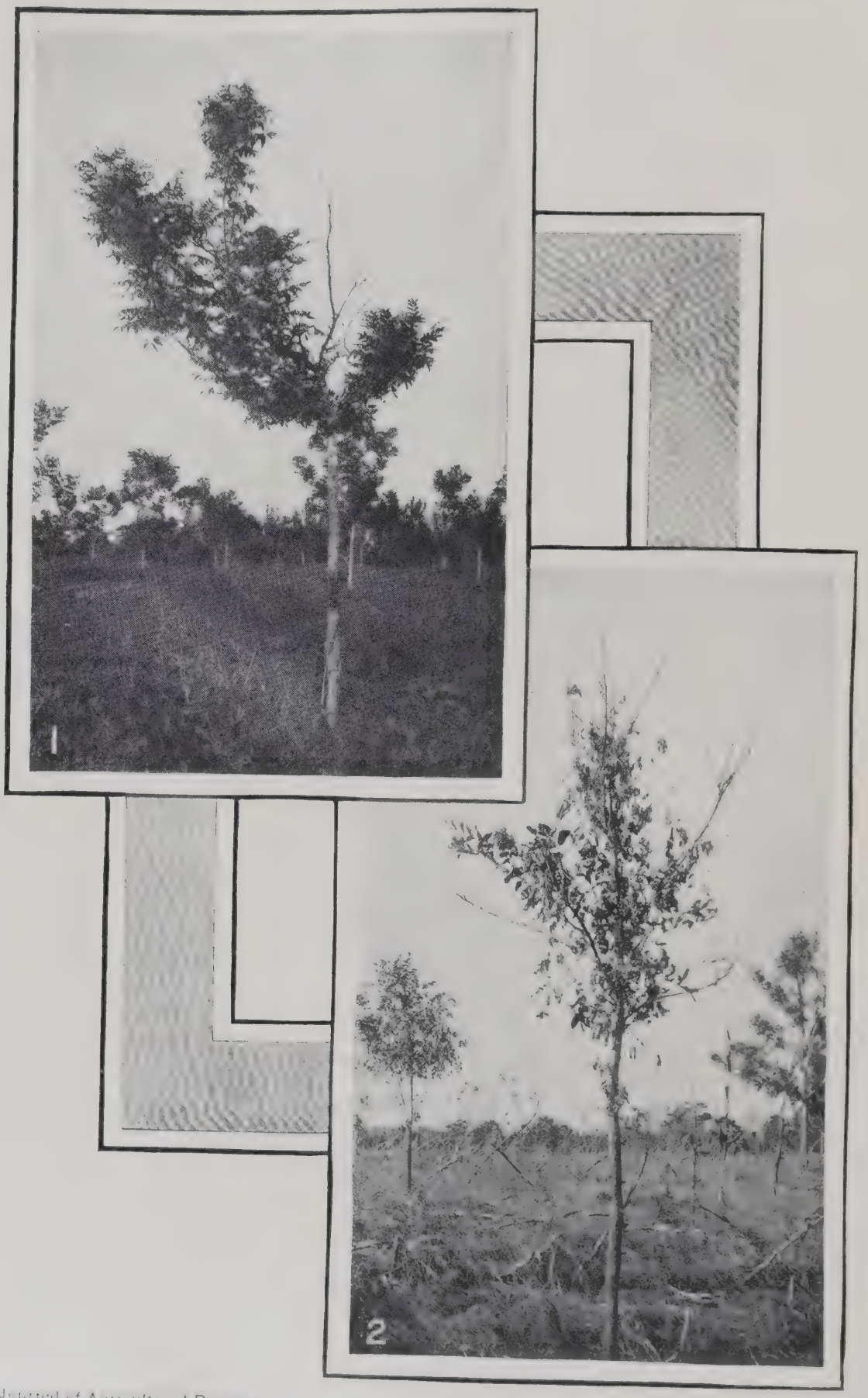
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PLATE XXVII

Fig. 1.—Young orchard pecan tree with a moderate attack of rosette on the left side and seriously dying back from the disease on the other side. Note the cover crop of cowpeas. From Cairo, Ga., November, 1905.

Fig. 2.—Young orchard pecan tree in advanced stages of rosette. Orchard interplanted with corn. From Blackshear, Ga., November, 1905.

PLATE XXVIII

Fig. 1.—Young orchard tree with severe attack of rosette. Note dying back of branches. Orchard interplanted with corn. From Blackshear, Ga., November, 1904.

Fig. 2.—Rosetted pecan tree cut off to the stump the preceding season, with the present season's growth again distinctly showing rosette. From Cairo, Ga., October, 1903.

Fig. 3.—Two seedling pecan trees planted the same day from the same lot of seedlings. The tree in the background was normal, while the one in the foreground had been affected with rosette almost from the time of planting. Note the effect of a severe and long-standing case of the disease. From Tallahassee, Fla., summer of 1912.

1
2
3

PRELIMINARY AND MINOR PAPERS

A NITROGENOUS SOIL CONSTITUENT: TETRACARBONIMID

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In 1913 brief mention (Shorey, 1913a)[1] was made of the isolation from a soil of a nitrogenous compound that had the properties of tetracarbonimid ($C_4H_4N_4O_4$). Since then this compound has been isolated from a number of soils and its identity as tetracarbonimid has been confirmed.

In the isolation of this compound an alkaline extract obtained by treating the soil with a 2 per cent solution of sodium hydroxid for 24 hours at room temperature was used. This extract was made acid with sulphuric acid and filtered. The acid filtrate was shaken out with ether to remove the acids and other compounds that are soluble in this solvent, and a solution of mercuric sulphate in dilute sulphuric acid was added until no further precipitate was formed. The precipitate was filtered off, well washed, suspended in water, and decomposed with hydrogen sulphid.

After the removal of mercuric sulphid by filtration, the dark-colored filtrate was concentrated to a smaller volume and excess of a solution of neutral lead acetate added. The dark-colored precipitate thus formed was removed by filtration and ammonia added to strong alkalinity. The white or cream-colored precipitate thus formed was filtered off, well washed, and decomposed by hydrogen sulphid, the lead sulphid was filtered off, and the filtrate was evaporated almost to dryness and allowed to stand. Crystals formed in this semisolid mass in a short time, and after standing a number of hours several volumes of alcohol were added and the solution was filtered. The alcoholic filtrate was concentrated to a small volume, and from this solution crystals separated in a short time. These were separated by filtration, dissolved in hot water, the solution filtered and concentrated to the crystallizing point and allowed to stand several hours, when the compound separated in a fairly pure form. By repeating this operation several times it was obtained in the form of small plates or prisms free from color.

Several points in this method as thus briefly outlined are deserving of further comment. The original acid filtrate from the humus extract is usually dark colored, and the treatment with mercuric sulphate in acid solution produces a dark-colored precipitate that removes nearly all color from the solution. When this precipitate is decomposed with hydrogen sulphid, a dark-colored solution again results from which neutral lead acetate precipitates most of the color without apparently removing the compound under discussion. This is mentioned because

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 178.

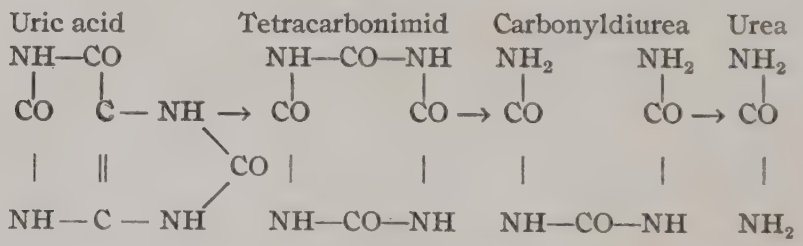
tetracarbonimid when alone and pure is precipitated by neutral lead acetate.

Further, when tetracarbonimid is pure, it is very slightly soluble in alcohol, but the presence of impurities which accompany it in this method of isolation affects its solubility in alcohol to such an extent that the first step in its purification is best effected by solution in alcohol. This treatment separates it from calcium salts and other inorganic impurities that always accompany it when precipitated from soil extracts by lead or mercury salts.

The crystals of the compound as first obtained are in the form of thin micaceous scales of rather unusual appearance, but since this changes when the compound is purified it can not be regarded as characteristic.

As thus obtained from soils, tetracarbonimid is in the form of small white prisms or plates rather difficultly soluble in cold but readily soluble in hot water. The crystals are very little soluble in alcohol, ether, or other solvents. The compound has no definite melting point, but when heated above 300° C. it decomposes, giving off acrid vapors that redden blue litmus paper, a white sublimate being deposited at the same time. An aqueous solution gives precipitates with silver, mercury, or lead salts. Barium chlorid produces no precipitate, but on the addition of barium chlorid and sodium hydroxid it is completely thrown down as a voluminous white precipitate.

Tetracarbonimid ($C_4H_4N_4O_4$) was first obtained by Scholtz (1901) by oxidizing uric acid in alkaline solution with hydrogen peroxid. The work of Scholtz was confirmed in 1909 by Schittenhelm and Wiener (1909), who by modifying the conditions of oxidation obtained another compound, carbonyldiurea ($C_3H_6N_4O_3$). These authors suggested that tetracarbonimid and carbonyldiurea might be intermediate steps in the oxidation of uric acid to urea in the human body, as shown below.



Tetracarbonimid was prepared from uric acid according to the method of Scholtz and its properties compared with the compound obtained from the soil. The two preparations were found to be identical in crystalline form and solubility, in being precipitated from solution by metallic salts, and in their behavior on heating. Their identity was further confirmed by the analysis of the barium salt and the determination of the nitrogen content.

The preparation from the soil was found on analysis to contain the following percentage of nitrogen: Sample No. 1, 32.59 per cent, and sample No. 2, 32.47 per cent of nitrogen, the theoretical nitrogen content of $C_4H_4N_4O_4$ being 32.61 per cent. The barium salt prepared by precipitating a hot, saturated solution of the soil compound with barium hydroxid was found to contain 61.55 per cent of barium, the barium content of $C_4Ba_2N_4O_4$ being 62 per cent. These figures, together with the correspondence in properties, are sufficient to establish the identity of the soil compound as tetracarbonimid.

Tetracarbonimid was first obtained from a sandy soil from Florida devoted to orange culture. This soil was one of the series of 16 samples referred to in a previous paper (1914), and in which vanillin and other benzene derivatives were found. Tetracarbonimid was found in three of these soils in sufficient quantity to make its identity certain, and in six other samples crystals having, so far as could be ascertained, the same properties as tetracarbonimid, were obtained in small quantities by the same method. This compound was also found in three out of four soils from other locations than Florida which were examined for it. These soils were (1) a sample of Norfolk sandy loam from Virginia, (2) a Sassafras soil from the grounds of the Department of Agriculture, and (3) a sample of Elkton silt loam from Maryland. These results indicate that tetracarbonimid is not an uncommon or accidental soil constituent.

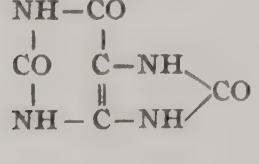
The soil from the Department grounds contained more of this compound than the others examined, and from 18 kg. of this soil 30 mg. of pure tetracarbonimid were obtained. The loss in purification in this separation was at least 50 per cent, so that, assuming that the treatment with sodium hydroxid extracted all of this compound, there was present per acre-foot of soil approximately 7 pounds of tetracarbonimid, representing 2.3 pounds of soil nitrogen. This soil had a total nitrogen content of 0.13 per cent, or approximately 5,200 pounds of nitrogen per acre-foot, and it appears that the quantity of tetracarbonimid nitrogen is at any one time but a very small part of the total.

However, in this investigation some evidence has been obtained indicating that the quantity of tetracarbonimid fluctuates under varying conditions of cultivation or crop growth. If, then, this compound is formed from other compounds under certain soil conditions and its disappearance by oxidation or other means, such as use by plants, is influenced also by conditions that vary, it may, in spite of the small quantity present at any one time, be an important step in the transformations that organic nitrogen undergoes in the soil.

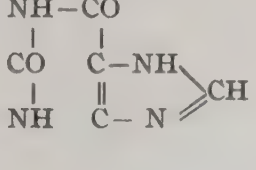
Tetracarbonimid has so far not been reported in any plant or animal tissue and has been made only by the mild oxidation of uric acid. Uric acid has not been found in soils or plants, and while it might be added to soils in human excrement, it is not a common constituent of manures or fertilizers; therefore it would seem that the source of tetracarbonimid in soils must be sought in some other compound.

The close relation of uric acid to the purin bases suggests these as possible sources of this compound, and there is no theoretical reason for assuming that any of the purin bases might not under proper conditions of oxidation yield tetracarbonimid. This possibility is disclosed more clearly by consideration of the structural formulas.

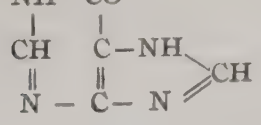
Uric acid
(2.6.8. oxypurin)



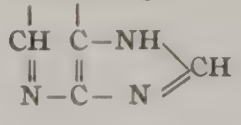
Xanthin
(2.6. oxypurin)



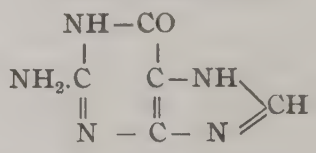
Hypoxanthin
(6 oxypurin)



Adenin
(6 amino-purin)



Guanin
(6 amino-6 oxypurin)



Xanthin, hypoxanthin (Schreiner and Shorey, 1910), and adenin (Shorey, 1913b, p. 16) have been found in soils, and guanin has been found in a soil that had been heated (Lathrop, 1912). All four bases have been found in plants and may also be formed from nucleic acid, a constituent of all nucleated cells of both plants and animals.

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APPLE ROOT BORER

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INTRODUCTION

While engaged in observations on the larval habits of the roundheaded apple-tree borer (*Saperda candida* Fab.) in West Virginia during the year 1911, the writer noticed in apple (*Malus* spp.) numerous burrows of some smaller insect associated with those of the former. Infested wood was collected and adults were reared, which were determined by Mr. E. A. Schwarz, of the Bureau of Entomology, as *Agrilus vittaticollis* Rand., a species of beetle which hitherto had not been recognized as an enemy of cultivated fruit trees. Further observations showed that the species is quite generally distributed throughout the Appalachian fruit region and that in places it is doing considerable damage to young apple trees.

The literature pertaining to this insect is meager. The species was first described from Massachusetts by J. W. Randall in 1837 and received the name which it now bears. In 1841 it was redescribed by Gory, in his monograph of the buprestids, as *Agrilus frenatus*. Le Conte, in 1857, lists the species as "*A. frenatus* Gory," with the note, "unknown to me." Again, in 1859 the same writer, in his Revision of the Buprestidae of the United States, uses both names as synonymous, giving neither the preference. In 1871 Edward Saunders in his Catalogus Buprestidarum, gives Randall's name the preference over Gory's. Since that time the species has been mentioned occasionally in lists of Buprestidae as *Agrilus vittaticollis* Rand.

E. P. Austin, in 1875, seems to have been the first to associate the insect with a host plant, at which time he notes: "Found occasionally in various parts of the State [Massachusetts] and seems to live on shadberry (*Amelanchier canadensis*)." In 1889 Frederick Blanchard, in his list of the Buprestidae of New England, records that in Massachusetts the beetle is found occasionally in June, feeding on the leaves of thorn, service, or shadbush (*Amelanchier canadensis*) and chokeberry. A specimen of the beetle in the United States National Museum was collected by W. F. Fiske in June at Tryon, N. C., on leaves of Oxydendrum. The species is also recorded from Michigan, Pennsylvania, and New Jersey, and it probably occurs throughout the greater part of the eastern United States.

At the suggestion of Prof. A. L. Quaintance, in charge of deciduous-fruit insect investigations for the Bureau of Entomology, a further study of the insect and its habits was made, the results of which are recorded below.

NATURE AND EXTENT OF INJURY

The injury to the trees is done by the slender, white larva of the insect, which bores through the sapwood and heartwood of the roots and lower trunk. The burrows through the roots frequently extend outward for

several feet and in badly infested trees are so numerous that the roots often die, causing a weakening of the whole tree.

The work of the insect is obscure, there being no chips or castings thrown to the surface, as is the case with the common roundheaded apple-tree borer. The egg, which is placed rather conspicuously on the bark of the trunk, and the exit hole in the bark through which the adult escapes from the wood, are the only external marks made by the insect on the tree. In addition to the injury resulting from the damaged roots, the exit holes in the bark admit more or less water, which frequently induces decay of the heartwood. The harm one individual root borer is capable of doing to a tree is much less than that usually done by a single roundheaded apple-tree borer, but in the localities where investigations were made the former species outnumbered the latter.

At French Creek, W. Va., 345 apple, pear, and service, or shadbush, trees, from ½ inch to 5 inches in diameter were cut off a few inches above the ground, and the burrows made by the larvæ of both species in ascending the trunks to pupate were counted. The three species of trees are favorite food plants of both of these borers, and the results of the counts, recorded in Table I, give an idea of the relative abundance of the two borers in the one locality. Similar results were obtained in several other localities where fewer trees were examined.

TABLE I.—*Relative abundance of burrows of Agrilus vittaticollis and Saperda candida in apple, pear, and service trees*

Kind of trees.	Number of trees.	Number of burrows of Agrilus.	Number of burrows of Saperda.
Apple	125	311	101
Pear	20	9	0
Service	200	342	21
Total	345	662	122

Most of the apple and pear trees cut for the foregoing counts were such seedlings as could be found in roadside fence corners and neglected orchards, and the service trees were in the woods. Only 36 of the 125 apple trees, or about 28 per cent, were free from the burrows of Agrilus and 87, or about 70 per cent, were free from burrows of Saperda.

FOOD PLANTS

The adult of this insect has been recorded by various writers as frequenting the foliage of service, wild thorn, and chokeberry. The writer has found the larva attacking apple, pear, wild thorn, wild crab, and service. It has been found rather abundantly in the following West Virginia localities: French Creek, Cherry Run, Sleepy Creek, Springfield, Moorefield, Romney, Keyser, Elkins, and Junior. Mr. E. B. Blakeslee, of the Bureau of Entomology, has found the larva in service trees at Winchester, Hayfield, and Staunton, Va. Of the several larval food plants named, apple and service seem to be greatly preferred above the others.

THE BURROWS

The burrows of the apple root borer (Pl. XXIX, figs. 1, 2, and 3) are of great length, that of a single larva often extending through the trunk and roots for 5 or 6 feet and in some cases even 8 feet. Throughout its length the burrow is packed with dustlike particles of wood that have passed through the alimentary canal of the insect. Cross sections of the burrows are oval in outline, those of full-grown larvæ being about 1 by 3 or 4 mm. in diameter.

Except for the discoloration of the dustlike packing, time does not change the appearance or form of the burrows in the wood until they are obliterated by decay. Fresh burrows are usually found within half an inch of the bark, and in large trees the position of a burrow in the wood gives some clue to its age. In the large number of old trees cut and examined during this investigation the finding of a large proportion of the burrows comparatively near to the bark is taken as an indication that this insect has been on the increase for the last 10 or 15 years.

LIFE HISTORY OF APPLE ROOT BORER

THE EGG

The eggs (Pl. XXXI, fig. 1) are somewhat variable in outline, an average specimen being flattened, oblong, disklike, 1.3 by 1.9 mm. in size. The surface is irregularly corrugated, the ridges and depressions being more distinct near the margin. When first laid, the egg is white or creamy white, but in a few days it changes to grayish brown, similar to the bark in color, and resembling in a general way the shield of a small scale insect. After the dark color is assumed, the embryo shows as a whitish spot at the center of the egg.

In the latitude of West Virginia the eggs are deposited in May and June. They are glued tightly to the bark of the trunk a few inches above the ground singly (Pl. XXX, fig. 3, *b*) or, rarely, in pairs. They are usually attached to a smooth surface, but are sometimes inserted into a crack or beneath a scale of bark. When the larva hatches, it leaves the egg from the underside and enters directly into the bark, thrusting its castings backward into the discarded shell and so filling it that it retains its normal size and shape. The abandoned shell often adheres to the bark for a year or longer.

THE LARVA

The larva (Pl. XXXI, fig. 2), before settling in the pupal chamber, is long, slender, distinctly segmented, flattened, and of nearly uniform width throughout, the first thoracic segment being slightly wider than the others. Full-grown specimens average from 3 to 4 by 30 to 36 mm. The color is white, except the small head and the anal forceps, which are brown. The anal forceps are prominent and shade from light brown at the base to dark brown or black at the converging tips. After entering the pupal cell, the larva contracts to about half its former length and in this condition (Pl. XXXI, fig. 3) it remains during the second winter of its life in the tree.

The larva lives in the tree for nearly two years, and during this time its movements are substantially as follows: On leaving the egg it bores

directly through the bark to the cambium, thence through the cambium down the trunk to the ground, whence it proceeds outward through a convenient root. After boring in this manner through the cambium for a distance of from 6 to 12 inches it burrows abruptly into the solid wood, where all the feeding throughout the remaining part of the larval stage is done. For most of its length the burrow through the cambium follows in a general way the grain of the wood. It sometimes zigzags across the grain directly after leaving the egg and invariably winds around the root in a spiral course once or twice just before leaving the cambium to enter the solid wood of the root. The burrow in the cambium soon heals and is scarcely discernible a year after it is made.

After burrowing into the solid wood of the root, the larva continues to feed outward from the tree. If the root is long enough, the burrow may continue toward the tip for a distance of 3 or 4 feet, after which it turns and is directed back toward the base. The larva spends its first winter well out from the trunk, often in a root not more than one-sixteenth of an inch in diameter. In penetrating these smaller roots it converts all the hard wood into powder during its outward and return journey. It is active late in the fall and early in the spring, and probably considerable feeding is done during the winter. With the coming of warm weather it feeds rapidly back toward the base of the root, and by midsummer it has reached the center of the root system and has begun to ascend the body of the tree. The latter part of the summer and the fall are spent in boring upward through the trunk (Pl. XXIX, fig. 2) and in fashioning a pupal chamber. In trees that are quite small pupation takes place within 5 to 10 inches of the ground, but in larger trees the larvæ for some reason ascend higher before forming the pupal cell. In apple and pear trees that are as large as 6 inches in diameter at the base of the trunk it is not unusual for the larvæ to ascend 2 or 3 feet to pupate, and in one case an individual was found in a 12-year-old pear tree that had burrowed up from the roots and pupated in a branch 46 inches above the ground.

The ascent through the trunk is usually made within half an inch of the inner bark, the larva occasionally approaching the bark but never entering it. The pupal chamber is a curved and enlarged terminus of the burrow, occupying a vertical position, with the convex side toward the heart of the tree. The upper end of the chamber curves abruptly to the bark, while at the lower end the curvature is more gradual and the chamber lacks one-sixteenth of an inch or more of extending to the bark. The two extremities are from 1 inch to 1½ inches apart, and when the chamber is completed both ends are packed with wood fragments, the insect occupying a space between.

In forming its pupal quarters the larva first extends a burrow of the usual transverse dimensions through to the bark at the upper end. It then recedes for about one-fourth of an inch and begins eating the wood from the side of the burrow. As this proceeds, the larva directs its excavation backward along the ventral side of its body and so doubles the diameter of the original gallery. The larva thus has its two ends folded closely together, and as the body moves forward the head and anus are soon contiguous. At this point the excavation ceases, and the head turns and follows the anus forward until both extremities are at the upper end of the chamber. Here the head rests, but the hinder

parts move downward until the axis of the body is again in line. At first the borer is much crumpled in accommodating itself to its restricted quarters, but a molt soon follows, after which all the segments of the body are so shortened that the space is adequate (Pl. XXX, fig. 1, *c*).

The larva does not get permanently settled in its cell until well into December, often after severe freezing weather has occurred.

THE PUPA

The pupa (Pl. XXXI, fig. 4) is broad and flattened dorsally, the males being smaller than the females. An average female measures 3.5 mm. wide by 12 mm. long. The color is white, the eyes, mouth parts, and, later, most of the abdomen and spots on the thorax changing to black as the imaginal stage is approached. The pupa occupies a vertical position in the cell, with its ventral side toward the bark (Pl. XXX, fig. 1, *b*).

Pupation takes place with the coming of the first few warm days of spring, occurring in the locality of the principal investigations from April 1 to 15. All individuals were found to have pupated in the valley of the Potomac River, near Moorefield and Springfield, W. Va., on April 20, 1912, while on the mountains a few miles distant, where the elevation was about 500 feet greater, no pupæ could be found on the same date. In Randolph County, West Virginia, at an altitude of about 2,500 feet above the sea, pupation had occurred on April 25, 1913. The pupal stage lasts from three to four weeks.

THE ADULT

The adult (Pl. XXXI, fig. 5) is a slender, elongate beetle, averaging 2.5 mm. wide by 10.5 mm. long. The elytra are black, usually with a tinge of purple; the head and thorax are iridescent purple or cupreous in some lights. The sides of the thorax and a wide stripe on top extending over the front of the head are covered with a dense bronze pubescence; the underparts are black, with a metallic purplish or coppery luster; the dorsal surface of the abdomen beneath the elytra is dark blue. A line of bronze pubescence extends along the sides of the abdomen and is visible dorsally beyond the inflected edge of the elytra. The front of the head is deeply impressed; the thorax is broader than long, its sides being regularly curved; the disk has oblique depressions on each side; the elytra are broadened behind the middle, the tips being separately rounded and finely serrulate. The upper surface is finely and densely granulate.

HABITS OF THE BEETLE

The adult apple root borers escape through small, round holes which they gnaw in the bark (Pl. XXX, fig. 3, *a*). They come forth in May, all the individuals of one generation issuing in a given locality during a comparatively short time. The beetles are active only by day, are rather quick to fly when disturbed, and have the buprestid habit of sunning themselves in exposed positions on bark and foliage.

It is probable that the life of the beetle does not often exceed two or three weeks. This is indicated by observations in the field and by experiences with a large number kept in wire cages placed over small apple trees. All beetles confined in such cages died within about two weeks.

HABITS OF OTHER MEMBERS OF THE GENUS AGRILUS

The apple root borer, *Agrilus vittaticollis*, belongs to a group that is represented by several well-known enemies of cultivated and forest plants and trees. The raspberry gouty-gall beetle (*Agrilus ruficollis* Fab.), the two-lined chestnut borer (*A. bilineatus* Weber), the bronzed birch borer (*A. anxius* Gory), and the sinuate pear borer (*A. sinuatus* Oliv.) are destructive pests whose food plants are indicated by their common names. The damage done by the different members of the genus is usually due to the larvæ working in or about the cambium, quite often of small branches or twigs. The root-feeding habit is not known to be common, although there are at least two or three other species in the Eastern States whose habits have not been fully described, the larvæ of which live in the roots of forest trees and shrubs.

NATURAL ENEMIES

So far as could be observed, the natural enemies of the apple root borer are confined to one species of hymenopterous parasite which attacks and destroys the larva and pupa (Pl. XXX, fig. 4). The adult of this parasite was first reared in April, 1912, and was described by Mr. H. L. Viereck[1] of the Bureau of Entomology, as representing a new genus, under the name *Xylophruridea agrili* (Pls. XXX, fig. 2, and XXXI, fig. 6). Two generations of this parasite occur annually, one brood of the adult appearing early in the spring and another late in the fall. Oviposition was not observed, but it is presumed that the female uses her strong external ovipositor to pierce the bark and wood, in order to get at her host. The spring brood of adult parasites oviposits on the root-borer pupæ or on the larvæ just before pupation, and the fall brood oviposits on the larvæ at about the time the pupal cell is being formed. The parasitic larvæ attack their host externally, those from the spring eggs developing rapidly, and, when full grown, constructing cocoons which occupy the host cell. This generation passes the greater part of the warm season of the year as larvæ in the cocoon. The larvæ from the fall brood of eggs develop less rapidly, some of them reaching full growth and constructing cocoons in the fall and early winter and others not entering the cocoon until very early in the spring.

The adult parasites have difficulty in escaping from their transforming quarters, and many of them die in their effort to gnaw their way out through the bark. Parasitization is much more frequent with the borers that are in very small trees. This is doubtless due to the fact that the borers in such trees are forced to work nearer the bark, where they are more constantly within reach of the ovipositor of the parasite. On the whole, from 25 to 40 per cent of the root borers are destroyed by this parasite.

REMEDIAL AND PREVENTIVE MEASURES

On account of the larva's concealed method of feeding, the digging-out process, which is used effectively against several species of fruit-tree borers, is not practicable or possible in this case. Control measures must be directed toward the protection of the trunk of the tree against the

[1] Viereck, H. L. Contributions to our knowledge of bees and ichneumon flies, including the descriptions of 21 new genera and 57 new species of ichneumon flies. Proc. U. S. Nat. Mus., v. 42, p. 646. 1912.

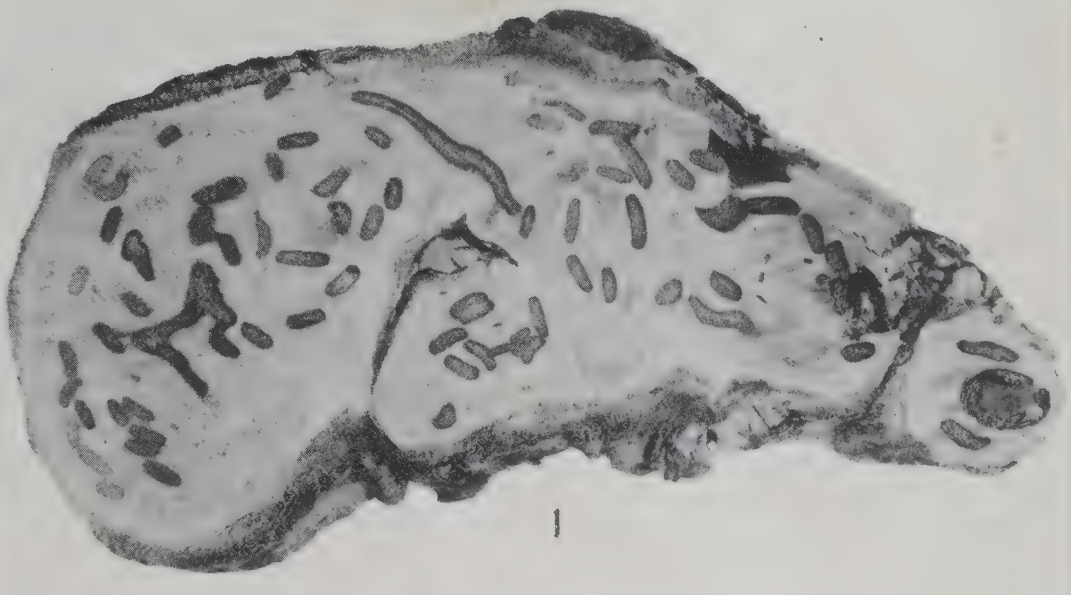
deposition of the egg rather than the killing of the borer after it begins feeding. Where paints, washes, or mechanical devices of any kind are used on trees as a preventive of injury by the roundheaded apple tree borer, equal protection may be had against the apple root borer by treating the trunks at about the time fruit is setting in the spring. The egg-laying season is of short duration, and temporary wrappers of paper or burlap, or any other material that will entirely cover the lower 2 feet of the trunk for a period of four or five weeks following the blooming season of apple, will in a large measure prevent eggs from being placed on the bark. Treatment with sticky adhesives or heavy paints that are not injurious to the trees will answer the same purpose.

The apple root borers develop freely in the common service tree, and the proximity to apple orchards of woods in which this tree flourishes may always be regarded as a source of possible infestation to the orchard.

PLATE XXIX

Figs. 1 and 3.—Sections of an apple root, showing burrows of the apple root borer (*Agrilus vittaticollis*). About natural size. Original.

Fig. 2.—Cross section of the trunk of a young apple tree, showing burrows made by the larvæ of the apple root borer in ascending the trunk to pupate. Natural size. Original.



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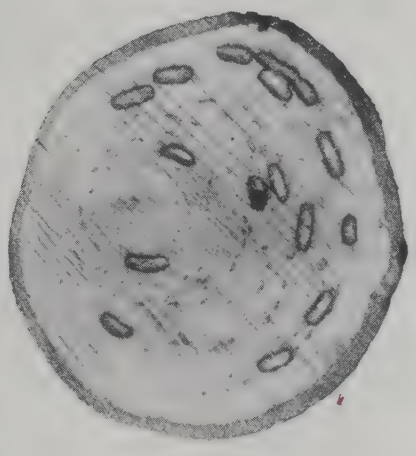

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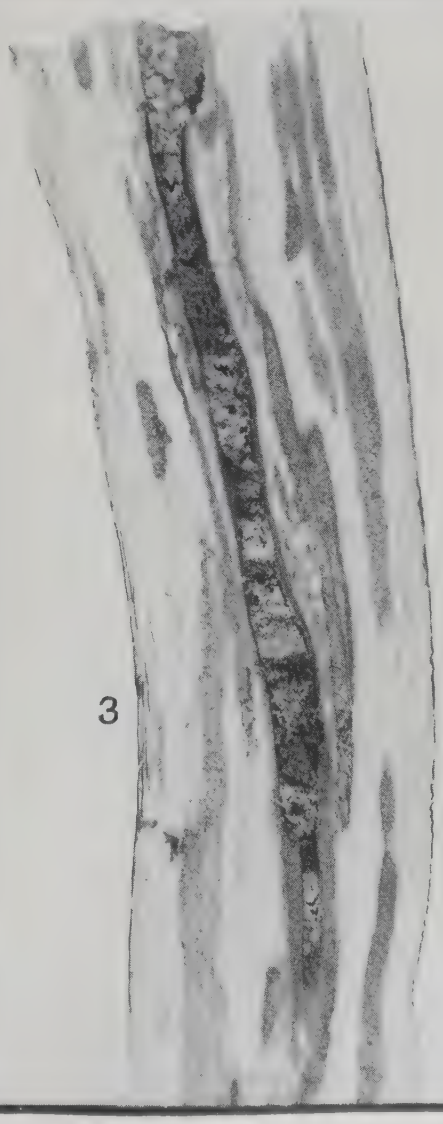

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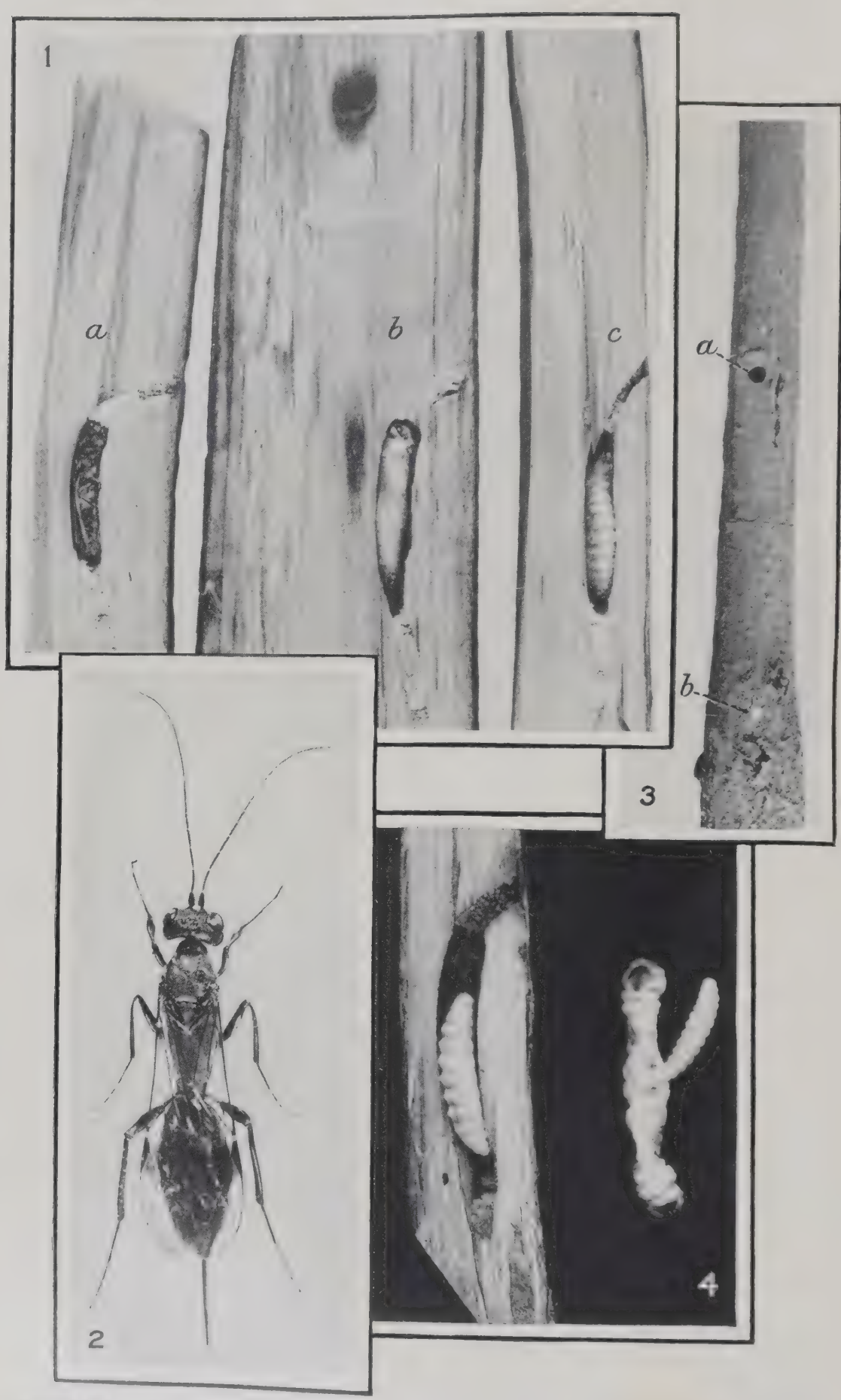
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PLATE XXX

Fig. 1.—*Agrilus vittaticollis:* Larva (*c*), pupa (*b*), and adult (*a*) of the apple root borer in the pupal cell. Slightly enlarged. Original.

Fig. 2.—*Xylophruridea agrili,* a common parasite of the apple root borer. Greatly enlarged; natural size, 18 mm. Original.

Fig. 3.—Section of trunk of young service tree, showing below the white egg and above the exit hole of the apple root borer. Natural size. Original.

Fig. 4.—*Xylophruridea agrili:* Larvæ of the parasite; one feeding on the larva and the other in the pupal cell of its host. Enlarged about one-third. Original.

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PLATE XXXI

Fig. 1.—*Agrilus vittaticollis:* Egg on trunk of young service tree. Original.
Fig. 2.—*Agrilus vittaticollis:* Feeding form of larva. × 4. Original.
Fig. 3.—*Agrilus vittaticollis:* Contracted form of larva as taken from pupal cell. × 6. Original.
Fig. 4.—*Agrilus vittaticollis:* Pupa. × 8. Original.
Fig. 5.—*Agrilus vittaticollis: a*, Adult, or beetle; *b*, claw; *c*, antenna. *a*, × 5; *b*, *c*, highly magnified. Original.
Fig. 6.—*Xylophruridea agrili*, a parasite of the apple root borer: Pupa. × 8. Original.

PLATE XXXI

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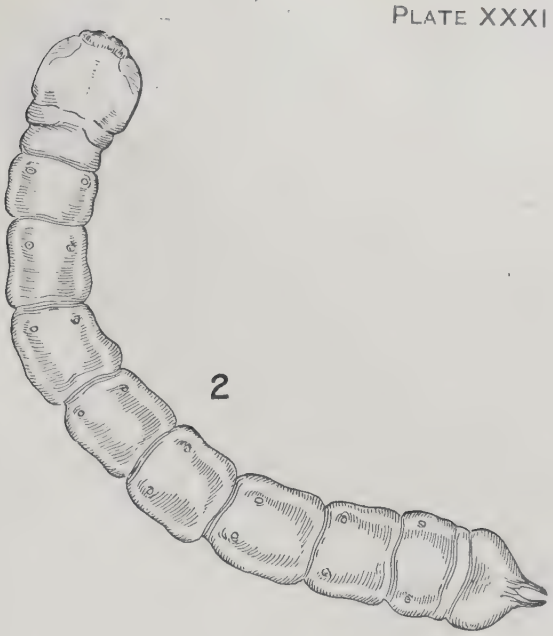

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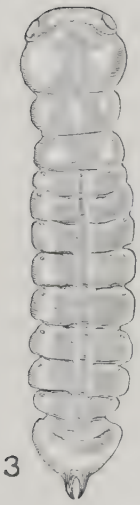

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CHANGES IN COMPOSITION OF PEEL AND PULP OF RIPENING BANANAS

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INTRODUCTION

In attempting to determine the effect of temperature on the rate of respiration of the banana (*Musa sapientum*), it was found that the rate of respiration increased very rapidly during ripening(13)[1], as, indeed, had been observed by Gerber (12), and the bananas maintained themselves during ripening at temperatures distinctly above that of their surroundings, in consequence of the intensely active respiration. The phenomenon of self-heating suggested the need of experiments in a calorimeter to determine the amount of heat evolved in relation to carbon dioxid formed and oxygen consumed. The necessity of accompanying such studies with analyses of samples of the bananas under observation led to the analytical work recorded in this paper.

The chemical changes which occur in the banana during ripening have been studied by Buignet (5); Corenwinder (8, 9, 10), Marcano and Muntz (17); Ricciardi (20); Doherty (11); Colby (7); Gerber (12); Balland (4); Atwater and Bryant (1); Chace, Tolman, and Munson (6); Bailey (2, 3); Prinsen Geerligs (18); Tallarico (21); Jähkel (14); Yoshimura (22); and Reich (19). The results have invariably been expressed in terms of the percentage of the pulp of the fruit when analyzed, and the data are, in consequence, on a constantly shifting basis, as the peel continuously loses weight and the weight of the pulp steadily increases. An accurate account of the chemical changes for use in exact biochemical studies was therefore lacking.

Four ripening experiments were made. In two experiments bunches of green bananas were ripened in a large respiration calorimeter designed for experiments with man. In the third and fourth experiments studies were made, in a specially designed ripening chamber, of the uniformity

[1] Reference is made by number to "Literature cited," pp. 202–203.

with which ripening occurs in different bunches of bananas and of the rate of starch hydrolysis during ripening in relation to changes in the rate of respiration. The first and second experiments were made in cooperation with Messrs. C. F. Langworthy and R. D. Milner, Nutrition Investigations, Office of Experiment Stations, who measured the weight changes on ripening, the carbon dioxid, the water and the heat evolved, and the oxygen absorbed. The composition was determined before and after ripening. Green bananas selected from commercial shipments just received from the West Indies were used in all cases.

FIRST EXPERIMENT

Six bunches of green bananas were used. They were evenly matured, so far as could be judged from the comparative intensity of the green color. The top and the bottom of the stem of each bunch were trimmed smoothly, and all injured fruits whose pulp might have been infected as a result of mechanical injury were removed.

For analysis, six fruits were cut from each of five bunches and eight fruits taken from the sixth bunch, which was larger than the others. The samples were taken from the inner and the outer portions of the "hands" at the top, the middle, and the bottom of each bunch, cutting them off where the short stem by which the bananas are attached to the stalks was most constricted. After ripening, samples were removed from the same "hands" from which samples had been previously taken, but they were cut from places removed from where the preceding samples had been attached, in order to avoid possible effects of local stimulus to adjacent fruits due to cutting. No indication of such effect, however, was noticeable.

The samples of green or ripe bananas were first weighed and then separated into peel and pulp. The peels of green bananas adhere closely to the pulp, and appreciable losses from evaporation are unavoidable. After separation, the peel and the pulp were weighed, to determine their respective proportions and the losses from evaporation. To express the analyses in terms of the original bananas, it was necessary to correct for this evaporation. To this end the loss in weight on peeling was arbitrarily divided equally between the respective weights of peel and pulp, and the percentages of peel and pulp were then calculated. The samples of peel and pulp were ground by passing them through a food chopper.

The methods of analysis used in this and succeeding studies were as follows:

SOLIDS were determined by evaporation in vacuo at 70° C.

ASH AND SOLUBLE ALKALINITY OF ASH were determined by the official methods.[1]

[1] Wiley, H. W., et al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr., Bur. Chem. Bul. 107 (rev.), p. 38, 102. 1908.

NITROGEN determinations were made by the official methods[1] by Mr. T. C. Trescot, of the Bureau of Chemistry.

ETHER EXTRACT was determined by exhausting with alcohol, and determining the ether extract in residue and alcohol-soluble extract. In case of the alcohol-insoluble extract the alcohol was evaporated, the residue transferred to a separatory funnel by use of water and ether, and extracted repeatedly with ether. These extracts were then combined, washed with water, and evaporated in tared flasks.

CARBOHYDRATES were determined by treating weighed samples with 80 per cent alcohol in Soxhlet extractors, using several portions of alcohol so as to avoid giving the larger proportion of the sugars a prolonged heat treatment. The residues were dried and weighed, and the weighed portions used in the estimation of starch and pentosans. The extracts were mixed with a little calcium carbonate, evaporated on the steam bath in a current of air, avoiding evaporation to dryness, and then taken up in water, treated with lead acetate, and made up to a known volume. After filtering, the excess of lead was removed by use of dry sodium oxalate.

REDUCING SUGAR AND SUCROSE were determined in this solution by applying the copper-reduction method of Munson and Walker,[2] before and after inversion.[3]

STARCH. To determine starch, 3-gram samples of the alcohol-insoluble material were transferred to 300 c. c. flasks, hydrolyzed with hydrochloric acid as directed in the official method,[4] and the dextrose determined by the copper-reduction method of Munson and Walker.[5]

PENTOSANS were determined in the alcohol-insoluble residues by the provisional method of the Association of Official Agricultural Chemists.[6]

Although at the time it was supposed that due precautions had been taken to prevent inversion of sucrose, unfortunately it is not improbable that more or less inversion may have occurred, owing to the failure to heat the alcohol extract to boiling.

When removed, the bananas were of a bright clear yellow, with a little green showing at the tips. They were entirely free from decay. The fruit surfaces were moist and waxy, indicating that the humidity had been high. During ripening, the calorimeter record showed that the temperatures had varied from 18.8° to 20.4° C., average 20.1° C. The oxygen content of the air supplied varied from 20.4 to 10.8 per cent by volume, average 15.5 per cent, probably sufficient for normal ripening.

The bananas weighed 137.71 kg. when placed in the calorimeter and 132.37 kg. when withdrawn, a loss in weight of 3.88 per cent. The

[1] Op. cit., p. 7.
[2] Op. cit., p. 241.
[3] Op. cit., p. 41.
[4] Op. cit., p. 53.
[5] Op. cit., p. 241.
[6] Op. cit., p. 54.

fruit consisted of 41.72 per cent of peel and 58.28 per cent of pulp when green and of 37.85 per cent of peel and 62.15 per cent of pulp when ripe. Calculated on the basis of the original bananas, the percentage of ripe peel was 36.38, a loss of 5.34 per cent of its weight. On the same basis the percentage of pulp was 59.73, a gain in weight of 1.45 per cent. Expressed in terms of the original bananas, the solids in the peel decreased from 5.21 to 4.88 per cent, the water from 36.51 to 31.50 per cent, about two-thirds of the starch passed into sugars, and the pentosans decreased slightly. The ash, the alkalinity of ash, the nitrogen, and the ether extract did not change materially. There was an apparent net loss in carbohydrates of 0.46 per cent.

Expressed in the same way, the solids in the pulp decreased from 16.93 to 16.74 per cent. The water increased from 41.35 to 42.99 per cent. The starch content changed from 13.15 to 2.40 per cent, the reducing sugars from 0.37 to 10.34 per cent, and the sucrose from 0.48 to 1.52 per cent. There was a net loss in carbohydrates of 0.88 per cent, due to respiration. The pentosans changed from 0.40 to 0.18 per cent. As in the peel, the ash, the alkalinity of ash, the nitrogen, and the ether extract in the pulp did not undergo marked changes in the amounts present. See Table I.

TABLE I.—*Composition of bananas before and after ripening in respiration calorimeter*

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF PEEL OR PULP WHEN ANALYZED

Part of fruit.	Percentage of whole fruit.	Composition.										
		Water.	Solids.	Ash.	Alkalinity of ash as K_2CO_3.	Protein, N × 6.25.	Ether extract.	Reducing sugar as invert.	Sucrose.	Starch.	Alcohol-insoluble solids.	Pentosans.
Peel:												
Green bananas	41.72	87.52	12.48	1.32	0.82	0.61	0.89	0.70	0.23	4.84	8.98	0.92
Ripe bananas	37.85	86.59	13.41	1.54	1.04	.64	1.08	3.71	.10	1.94	6.24	.89
Pulp:												
Green bananas	58.28	70.95	29.05	.87	.49	1.20	.24	.64	.83	22.56	26.28	.69
Ripe bananas	62.15	71.98	28.02	.85	.47	1.24	.24	17.31	2.54	4.02	6.36	.30

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF THE WHOLE GREEN BANANAS [a]

Peel:												
Green bananas	41.72	36.51	5.21	0.55	0.34	0.26	0.37	0.29	0.10	2.02	3.75	0.38
Ripe bananas	36.38	31.50	4.88	.56	.38	.23	.39	1.35	.04	.71	2.27	.32
Pulp:												
Green bananas	58.28	41.35	16.93	.51	.29	.70	.14	.37	.48	13.15	15.32	.40
Ripe bananas	59.73	42.99	16.74	.51	.28	.74	.14	10.34	1.52	2.40	3.80	.18

[a] Percentage of loss in weight on ripening, 3.88.

SECOND EXPERIMENT

In the second experiment similar composition changes occurred. In addition, it was demonstrated that the banana stem does not contribute materially to the fruit during ripening. The details of this experiment are as follows:

Four bunches of green bananas, consisting of the most evenly ripened fruit, judging by color, of a lot of eight bunches of the green fruit, were placed in the calorimeter after sampling and weighing. Six fruits had been removed as samples from each of two bunches of green bananas and eight from each of the others.

During ripening the temperature varied from 20.9° to 24.2° C., averaging 23.1° C.; the oxygen content of the air varied between 20.0 and 6.1 per cent by volume, averaging 14.1 per cent; and the humidity remained high, as before. When removed, all of the fruits were thoroughly ripe, and indeed one bunch was slightly overripe. Several specimens on this bunch were beginning to spoil. The skins were bright golden yellow, and the fruit surfaces were waxy and moist. No browning occurred, except where the bananas were superficially injured.

After weighing, the bunches were again sampled. In the analyses determinations of protein, ash, and ether extract were omitted, as the quantities of these substances present had been found to change but very slightly during ripening.

The four bunches remaining after selection of the bananas for the respiration calorimeter were used in the study of the composition of the green stem. Mr. W. H. Evans, of the Office of Experiment Stations, suggested that possibly the stem contained reserve materials supplied to the fruit during ripening. The bananas were detached from the stems in the same manner as when taking samples for analysis, leaving on the stems the stubs of the short stems by which the bananas were attached. The percentage of stems was 5.04 per cent. They were finely divided in a shredding machine and analyzed, using the methods employed for the analysis of peel and pulp. At the conclusion of the ripening experiment in the calorimeter the weight of the stems of the ripened bananas was determined and the stems then ground and analyzed.

The original weight of the four bunches of green bananas placed in the calorimeter after sampling was 75.90 kg. They suffered a loss upon ripening of 6.36 kg., or 8.38 per cent.

The bananas after ripening consisted of 95.30 per cent of fruit and 4.70 per cent of stem. Assuming that the fruit and stalks lost weight in equal proportions, the respective weights of green fruits and stems when placed in the calorimeter were 72.33 and 3.57 kg. The proportions of pulp and peel of the green bananas were 58.22 and 41.78 per cent, respectively. The entire bunches of green bananas as placed in the calorimeter after sampling consisted therefore of 55.48 per cent of

pulp, 39.82 per cent of peel, and 4.70 per cent of stem. When ripe, the bunches consisted of 63 per cent of pulp, 32.30 per cent of peel, and 4.70 per cent of stem. Based, however, upon the weight of the original bunches of green bananas, the proportion in ripe bananas was 57.72 per cent of pulp, 29.59 per cent of peel, and 4.32 per cent of stem, a total of 91.63 per cent.

The analytical data are given in Table II in terms of percentage of pulp and peel as analyzed (corrected for loss in weight in peeling), in terms of the original whole bananas, and in terms of the entire bunch of bananas—i. e., including the stem. The ripening period was longer than in the first experiment in the calorimeter, more starch disappeared, and more sugars formed, while the gains of water in pulp and losses in peel were greater. The study of the composition of the stem before and after ripening showed that the changes which occur in it during the ripening process are so slight as to be insignificant. The percentage of loss in solids, on the basis of the original bananas, was but 0.04 per cent.

TABLE II.—*Composition of bananas before and after ripening in respiration calorimeter*

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF PEEL, PULP, AND STEM OF THE BANANAS BEFORE AND AFTER RIPENING

Part of fruit.	Percentage of whole fruit.	Composition.						
		Water.	Solids.	Reducing sugar as invert.	Sucrose.	Starch.	Alcohol-insoluble solids.	Pentosans.
Peel:								
Green bananas.....	41.78	88.28	11.72	0.76	0.02	4.43	8.55	0.86
Ripe bananas.....	33.89	85.12	14.88	4.42	.12	1.65	6.77	.96
Pulp:								
Green bananas.....	58.22	70.76	29.24	.71	.02	24.10	27.03	.68
Ripe bananas.....	66.11	73.00	27.00	13.81	8.50	1.17	3.42	.25
Stem:								
Green bananas.....	[a] 4.70	90.19	9.81	.63	.17	1.59	7.02	.....
Ripe bananas.....	4.70	88.97	11.03	.30	.04	1.83	8.72	.....
COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF THE WHOLE GREEN BANANAS [b]								
Peel:								
Green bananas.....	41.78	36.88	4.90	0.32	0.01	1.85	3.57	0.36
Ripe bananas.....	31.05	26.43	4.62	1.37	.04	.51	2.10	.30
Pulp:								
Green bananas.....	58.22	41.20	17.02	.41	.01	14.03	15.74	.40
Ripe bananas.....	60.57	44.22	16.35	8.37	5.15	.71	2.07	.15
COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF THE WHOLE STEM OF GREEN BANANAS [b]								
Peel:								
Green bananas.....	39.82	35.15	4.67	0.30	0.008	1.76	3.40	.....
Ripe bananas.....	29.59	25.19	4.40	1.31	.04	.49	2.00	.. ..
Pulp:								
Green bananas.....	55.48	39.26	16.22	.39	.01	13.37	15.00	.....
Ripe bananas.....	57.72	42.14	15.58	7.97	4.91	.68	1.97	.....
Stem:								
Green bananas.....	4.70	4.18	.52	.03	.01	.08	.33	.. ..
Ripe bananas.....	4.32	3.84	.48	.01	.002	.08	.38	.. ..

[a] It is here assumed that the same proportion of stem is present in the green as in the ripe bananas.
[b] Percentage of loss in weight on ripening, 8.38.

THIRD EXPERIMENT

It now seemed well to observe the composition of the banana of commerce repeatedly at several stages during its ripening, in order to determine the uniformity of the changes which occur in bananas from different bunches. Four bunches of bananas were ripened in a specially constructed humidity chamber and each bunch was sampled three times during its ripening. This experiment demonstrated that the changes in ripening in the four bunches were remarkably uniform, while the data secured in the first two experiments were repeatedly confirmed. In addition, the study gives a good idea of the composition of bananas when just ripe enough to be edible and also when very ripe.

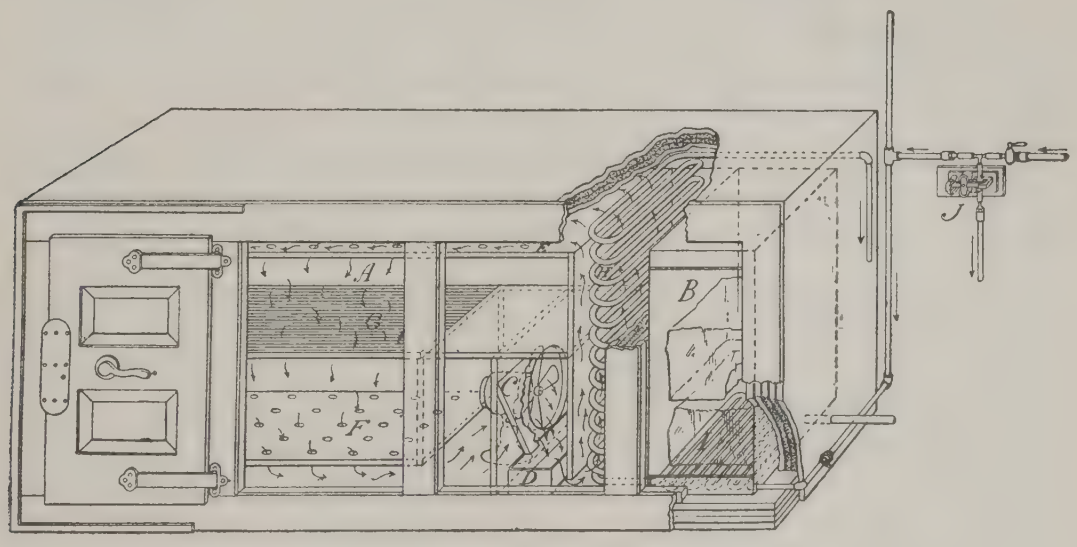

FIG. 1.—Constant-temperature humidor.

The detailed account of the experiment is as follows:

The humidity chamber (fig. 1) consisted of a large case, of cold-storage construction, divided by an insulated wall into two compartments, A and B. Compartment A was so arranged that it could be kept at constant temperature and humidity at temperatures below that of the room. Ice was placed in compartment B. In A the air was circulated continuously by an electric fan. The course of the air current was as follows: From the fan it was deflected by the baffle C to play upon the surface of water in the tank D. The water was here kept at constant level by a device not shown and was warmed by an electrically heated immersion heating coil, also not shown. The air then passed up through the vertical cooling coil H. The air, moisture laden and cooled, was then delivered through holes in the false ceiling E to the rest of the compartment, whence it was drawn back to the fan through holes in the false bottom F. Detached bananas or other objects were placed on the false bottom F or on the slatted shelf G. When bunches of bananas were being ripened, shelf G was removed and the fruit was suspended from hooks in the false ceiling.

Compartment B was lined with galvanized iron. At the bottom the horizontal coil I was connected through the partition with the vertical coil H in compartment A. The ice rested directly upon coil I. To cool compartment A, tap water was supplied to coil I and the ice-cold water displaced in coil I passed into coil H. Automatic delivery of cold water to coil H was accomplished by the operation of the sounder J, which in turn was controlled by the thermostat (not shown) in compartment A. The form of the thermostat has already been illustrated (13). The low-tension circuit opened and closed by the thermostat operated a relay in which a 110-volt D. C. circuit was opened and closed. In the 110-volt D. C. circuit a 32-candlepower carbon-filament lamp and the sounder J were placed in series. When the circuit was closed, the lever of the sounder compressed a rubber tube through which a slow stream of tap water was passing to waste. The water was then delivered to coil I. The sounder was of a resistance of 4 ohms.

The constancy of the apparatus is from 0.1° to 0.2° C., depending upon a number of factors, of which the external temperature and the rate of water flow are perhaps the most important. The humidity remained high and constant. The apparatus is capable of operating continuously for weeks at a time in the neighborhood of 20° C. in hot weather, with little or no attention, except that of being supplied with ice. The cold-storage features of the humidity chamber, without which it would be of little value for the purpose of operating at temperatures below that of the room, were suggested by Mr. S. J. Dennis, of the Bureau of Plant Industry.

Four large bunches of average-sized green bananas, carefully trimmed and sampled, were weighed and suspended in the humidity chamber and the bananas allowed to ripen. When their color had just changed from green to yellow, the tips of the fruit being still green, each lot was weighed, sampled as before, again weighed, and allowed to ripen further. The temperature was kept at 20° C. during ripening, and the air in the humidor was constantly renewed. When the bunches had become very ripe, most of the fruits showing slight superficial browning, the bunches were weighed and samples again taken. The pulp of the bananas was now soft, tender, and somewhat mealy. It was entirely free from translucent semiliquid portions and from decay. On weighing the bunches at this time many fruits broke off, and the experiment was discontinued.

The records of the weights permit reference of the results to the basis of the original bunches, assuming, as in the previously described studies, that the stems lost weight at the same rate as the bananas themselves. The analytical data are shown in Table III. In all cases nearly all of the starch of the peel and the pulp gradually passed into sugar. For completeness the ash, the alkalinity of the ash, and the protein were determined in both pulp and peel. As in the previous study, the changes

in the quantities of these ingredients present were insignificant. In the peel the transformation of starch into soluble carbohydrates was more rapid during the change from green to yellow than subsequently. In the pulp also the transformation of starch was the most rapid during the change in color of the bananas from green to yellow. By the end of this period about two-thirds of the starch had passed into soluble carbohydrates. During the subsequent ripening in each case a large proportion of the remaining starch in the pulp became converted into sugars. Some variation appears in the amounts of sucrose and invert sugar formed on ripening in the pulp of the bananas from the several bunches, but unfortunately a defect in the method existed (see p. 189), and more or less inversion of sucrose may have occurred during analysis. The figures for sucrose are probably slightly high and those for reducing sugars correspondingly low. Striking features of each set of analyses are the water changes. The peels lost water at uniform rates; the pulps all gained water—most rapidly after turning yellow.

TABLE III.—*Composition of bananas during ripening on the stem in the humidity chamber*

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGES OF PEEL AND PULP OF THE BANANAS BEFORE AND AFTER RIPENING

Serial No.	Part of fruit.	Interval of keeping in humidity chamber.	Loss in weight on ripening.	Percentage of whole fruit.	Composition of peel or pulp.								
					Water.	Solids.	Ash.	Alkalinity of ash as K_2CO_3.	Protein.	Reducing sugar as invert.	Sucrose.	Starch.	Alcohol-insoluble solids.
		Days.											
1065	Peel:												
	Green bananas....	0	0.00	40.30	89.06	10.94	1.45	0.95	0.64	0.90	0.23	4.20	8.31
	Ripe bananas.....	6	4.46	36.76	87.64	12.36	1.71	1.10	.68	3.26	.06	2.00	6.86
	Full ripe bananas.	13	10.04	31.15	84.31	15.69	2.09	1.38	.87	3.82	.34	1.98	8.30
	Pulp:												
	Green bananas....	0	0.00	59.70	72.94	27.06	.90	.53	1.04	.77	.37	21.54	24.88
	Ripe bananas.....	6	4.46	63.24	72.94	27.06	.94	.52	.97	16.41	1.02	5.42	7.92
	Full ripe bananas.	13	10.04	68.85	75.98	24.02	.86	.49	.93	13.96	5.35	.76	2.89
1066	Peel:												
	Green bananas....	0	0.00	40.15	87.75	12.25	1.37	.86	.92	.69	.21	4.94	9.49
	Ripe bananas.....	5	3.33	37.60	87.09	12.91	1.55	.95	.88	3.14	.51	2.54	7.52
	Full ripe bananas.	13	9.43	31.22	83.59	16.41	1.91	1.20	1.07	4.77	.26	1.94	8.08
	Pulp:												
	Green bananas....	0	0.00	59.85	72.21	27.79	.88	.52	1.30	.64	.32	22.55	25.92
	Ripe bananas.....	5	3.33	62.40	71.76	28.24	.90	.50	1.25	7.69	9.37	6.49	9.00
	Full ripe bananas.	13	9.43	68.78	75.70	24.30	.82	.47	1.16	14.55	5.07	.75	2.76
1067	Peel:												
	Green bananas....	0	0.00	40.35	88.13	11.87	1.37	.87	.88	.96	.12	4.42	8.74
	Ripe bananas.....	5	3.17	37.45	87.32	12.68	1.52	.96	.79	2.81	.65	2.33	7.09
	Full ripe bananas.	13	8.90	30.80	83.53	16.47	1.96	1.23	1.21	4.50	.19	1.98	8.33
	Pulp:												
	Green bananas....	0	0.00	59.65	72.12	27.88	.92	.54	1.37	.41	.51	22.43	26.05
	Ripe bananas.....	5	3.17	62.55	71.87	28.13	.95	.50	1.32	7.36	9.17	6.56	8.95
	Full ripe bananas.	13	8.90	69.20	75.40	24.60	.86	.48	1.28	13.12	6.70	.77	2.78
1068	Peel:												
	Green bananas....	0	0.00	43.44	88.74	11.26	1.32	.86	.73	.96	.15	4.10	8.33
	Ripe bananas.....	7	3.98	39.70	87.64	12.36	1.51	.98	.74	2.96	.11	2.06	6.62
	Full ripe bananas.	13	8.02	34.27	85.38	14.62	1.83	1.20	.93	3.56	.16	1.77	7.49
	Pulp:												
	Green bananas....	0	0.00	56.56	71.90	28.10	.85	.49	1.20	.29	.43	22.51	26.22
	Ripe bananas.....	7	3.98	60.30	72.07	27.93	.90	.48	1.14	14.69	1.98	6.88	9.66
	Full ripe bananas.	13	8.02	65.73	74.92	25.08	.80	.45	1.06	9.91	6.81	1.01	3.18

TABLE III.—*Composition of bananas during ripening on the stem in the humidity chamber*—Continued

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF THE WHOLE GREEN BANANAS

Serial No.	Part of fruit.	Interval of keeping in humidity chamber.	Loss in weight on ripening.	Percentage of whole fruit.	Composition of peel or pulp.								
					Water.	Solids.	Ash.	Alkalinity of ash as K_2CO_3.	Protein.	Reducing sugar as invert.	Sucrose.	Starch.	Alcohol-insoluble solids.
	Peel:	*Days.*											
	Green bananas....	0	0.00	40.30	35.89	4.41	0.58	0.38	0.26	0.36	0.09	1.69	3.35
	Ripe bananas.....	6	4.46	35.12	30.78	4.34	.60	.39	.24	1.15	.02	.70	2.41
1065	Full ripe bananas.	13	10.04	28.02	23.63	4.40	.59	.39	.24	1.07	.10	.56	2.33
	Pulp:												
	Green bananas....	0	0.00	59.70	43.54	16.15	.54	.32	.62	.46	.22	12.86	14.85
	Ripe bananas.....	6	4.46	60.42	44.07	16.36	.57	.31	.59	9.92	.62	3.27	4.79
	Full ripe bananas.	13	10.04	61.94	47.06	14.88	.53	.30	.58	8.65	3.31	.47	1.79
	Peel:												
	Green bananas....	0	0.00	40.15	35.23	4.92	.55	.35	.37	.28	.08	1.98	3.81
	Ripe bananas.....	5	3.33	36.35	31.66	4.69	.56	.35	.32	1.14	.18	.92	2.73
1066	Full ripe bananas.	13	9.43	28.28	23.64	4.64	.54	.34	.30	1.35	.07	.55	2.29
	Pulp:												
	Green bananas....	0	0.00	59.85	43.22	16.63	.53	.31	.78	.38	.19	13.50	15.51
	Ripe bananas.....	5	3.33	60.32	43.35	17.05	.54	.30	.76	4.64	5.66	3.92	5.43
	Full ripe bananas.	13	9.43	62.29	47.16	15.14	.51	.29	.72	9.06	3.16	.47	1.72
	Peel:												
	Green bananas....	0	0.00	40.35	35.56	4.79	.55	.35	.36	.39	.05	1.78	3.53
	Ripe bananas.....	5	3.17	36.26	31.66	4.60	.55	.35	.29	1.02	.24	.85	2.57
1067	Full ripe bananas.	13	8.90	28.06	23.44	4.62	.55	.35	.34	1.26	.05	.56	2.34
	Pulp:												
	Green bananas....	0	0.00	59.65	43.02	16.63	.55	.32	.82	.25	.30	13.38	15.54
	Ripe bananas.....	5	3.17	60.57	43.53	17.04	.58	.30	.80	4.46	5.55	3.97	5.42
	Full ripe bananas.	13	8.90	63.04	47.53	15.51	.54	.30	.78	8.27	4.22	.49	1.75
	Peel:												
	Green bananas....	0	0.00	43.44	38.55	4.89	.57	.37	.32	.42	.07	1.78	3.62
	Ripe bananas.....	7	3.98	38.12	33.41	4.71	.58	.37	.28	1.13	.04	.79	2.52
1068	Full ripe bananas.	13	8.02	31.52	26.91	4.61	.58	.38	.29	1.13	.05	.56	2.36
	Pulp:												
	Green bananas....	0	0.00	56.56	40.67	15.89	.48	.28	.68	.16	.24	12.73	14.83
	Ripe bananas.....	7	3.98	57.90	41.73	16.17	.52	.28	.66	8.50	1.15	3.98	5.59
	Full ripe bananas.	13	8.02	60.46	45.30	15.16	.48	.27	.64	5.99	4.12	.61	1.92

A statement of the average composition of the pulps of the four bunches of bananas as analyzed, summarized from Table III, is given in Table IV.

TABLE IV.—*Average composition in terms of percentage of pulp of bananas at different stages*

Stage.	Percentage of whole fruit.	Water.	Solids.	Ash.	Alkalinity of ash.	Protein, N×6.25.	Reducing sugars as invert.	Sucrose.	Starch.
Green pulp.....	58.94	72.29	27.71	0.89	0.52	1.23	0.53	0.41	22.26
Ripe pulp.....	62,12	72.16	27.84	.92	.50	1.17	11.54	5.39	6.34
Very ripe pulp.....	68.14	75.50	24.50	.84	.47	1.10	12.89	5.98	.82

FOURTH EXPERIMENT

In the fourth experiment lots of bananas of the same initial ripeness and taken from the same stem were analyzed successively during ripening. The rate of respiration of each lot was determined immediately before its analysis. It was thus possible to correlate the chemical

changes with the changes in rate of respiration. The results show that the most rapid starch hydrolysis occurs at the time when the respiration rate is greatest. The following is a detailed account of the work.

The fruits from a large bunch of green bananas were cut from the stem by first cutting off the "hands" and then separating the individual fruits, finally removing from each fruit the small piece of adhering stem, cutting where the stem was most constricted, as in sampling bananas in previous studies. A few drops of sticky, turbid juice oozed from each banana, and care was taken not to allow the fruit surfaces to become wet with it. Fruits from the inside and the outside of each "hand" were divided as evenly as practicable into nine different lots, each lot receiving fruits from all parts of the bunch, and consisting of from 13 to 17 fruits. The lots were weighed and placed in the humidity chamber on the afternoon of May 1. The analysis of the first lot was made upon the afternoon of the following day, immediately after the determination of its respiratory activity. The remaining lots were successively analyzed at intervals of from one to three days, except the ninth lot, which was kept longest, and spoiled. See Table V.

TABLE V.—*Composition of detached bananas during ripening in the humidity chamber*

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGES OF PEEL AND PULP OF THE BANANAS BEFORE AND AFTER RIPENING

Date.	Part of fruit.	Interval in humidity chamber.	Loss in weight in humidity chamber.	Percentage of whole fruit.	Composition of peel or pulp.				
					Water.	Solids.	Alcohol-insoluble solids.	Total sugar as invert.	Starch.
	PEEL.								
1912.		*Days.*							
May 2	Green bananas	1	1.66	38.22	89.46	10.54	6.83	1.39	3.28
3	Slightly yellowing bananas.	2	2.47	37.54	88.61	11.39	8.52	2.16	2.93
4	.....do	3	3.67	35.41	88.64	11.36	6.18	2.91	2.11
6	Yellow bananas	5	6.48	32.57	86.84	13.16	6.09	3.64	1.63
8	.....do	7	7.83	30.88	85.90	14.10	6.67	3.89	1.69
10	.....do	9	8.39	29.36	85.08	14.92	7.08	3.98	1.82
13	.....do	12	10.56	27.95	84.25	15.75	7.57	4.07	1.88
16	Brown bananas	15	11.32	26.26	83.32	16.68	8.12	4.38	1.84
	PULP.								
2	Green bananas	1	1.66	61.77	72.47	27.53	21.95	4.35	19.01
3	Slightly yellowing bananas.	2	2.47	62.45	72.37	27.63	16.46	9.65	13.96
4	.....do	3	3.67	64.58	72.44	27.56	11.04	15.00	8.62
6	Yellow bananas	5	6.48	67.42	73.26	26.74	5.82	19.65	3.66
8	.....do	7	7.83	69.12	73.45	26.55	3.89	20.08	1.73
10	.....do	9	8.39	70.65	74.63	25.37	3.13	20.61	1.15
13	.....do	12	10.56	72.05	75.72	24.28	2.88	20.05	.87
16	Brown bananas	15	11.32	73.74	77.02	22.98	3.17	18.21	1.01

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF THE WHOLE GREEN BANANAS

	PEEL.								
May 2	Green bananas	1	1.66	37.59	33.63	3.96	2.57	0.53	1.23
3	Slightly yellowing bananas.	2	2.47	36.61	32.44	4.17	3.12	.80	1.07
4	.....do	3	3.67	34.11	30.23	3.88	2.11	.99	.72
6	Yellow bananas	5	6.48	30.46	26.45	4.01	1.86	1.11	.50
8	.....do	7	7.83	28.46	24.45	4.01	1.90	1.11	.48
10	.....do	9	8.39	26.90	22.89	4.01	1.90	1.07	.49
13	.....do	12	10.56	25.00	21.06	3.94	1.89	1.02	.47
16	Brown bananas	15	11.32	23.29	19.40	3.89	1.89	1.02	.43

TABLE V.—*Composition of detached bananas during ripening in the humidity chamber*—Continued

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF THE WHOLE GREEN BANANAS—continued

Date.	Part of fruit.	Interval in humidity chamber.	Loss in weight in humidity chamber.	Percentage of whole fruit.	Composition of peel or pulp.				
					Water.	Solids.	Alcohol-insoluble solids.	Total sugar as invert.	Starch.
	PULP.								
1912.		*Days.*							
May 2	Green bananas	1	1.66	60.74	44.02	16.72	13.33	2.65	11.55
3	Slightly yellowing bananas.	2	2.47	60.91	44.08	16.83	10.03	5.88	8.50
4	.....do	3	3.67	62.21	45.06	17.15	6.87	9.33	5.36
6	Yellow bananas	5	6.48	63.05	46.19	16.86	3.67	12.39	2.32
8	.....do	7	7.83	63.70	46.79	16.91	2.48	12.79	1.10
10	.....do	9	8.39	64.72	48.30	16.42	2.03	13.34	.74
13	.....do	12	10.56	64.44	48.79	15.65	1.86	12.91	.56
16	Brown bananas	15	11.32	65.39	50.36	15.03	2.07	11.91	.65

In order to determine correctly the respiration rate at intervals on ripening, it was necessary to collect the carbon dioxid evolved during relatively short periods.

The bananas were placed in a tubulated desiccator, kept in the dark at 20° C., and a rapid current of air passed through. The air was first freed from carbon dioxid by passing it through a long wide glass tube filled with soda lime. The carbon dioxid evolved by the bananas was collected by drawing the air from the desiccator through a Reiset scrubbing tube containing soda solution. The Winkler method of titration of the absorbed carbon dioxid was employed.

In operating the flask of the Reiset apparatus was charged with a mixture of 500 c. c. of distilled water and 100 c. c. of an approximately normal solution of sodium hydroxid. The distilled water had previously been well aerated to remove carbon dioxid, and the titer of the soda solution (after addition of barium chlorid to precipitate carbonates) was known. After mixing, the Reiset tube was inserted, connection made with the desiccator containing the bananas, and suction applied. After absorption, the contents of the Reiset apparatus were washed into a large precipitating jar with aerated distilled water, excess of barium chlorid added, and the solution titrated with normal hydrochloric acid, using phenolphthalein as indicator. Each cubic centimeter of normal alkali consumed equals 0.022 gm. of carbon dioxid. In titrating it was necessary to admit the acid under the surface of the solution and stir well to avoid escape of carbon dioxid freed by local momentary excess of acid. As the amounts of carbon dioxid expected were approximately known it was found convenient to use a slight excess of solution of barium chlorid of such strength that each cubic centimeter decomposes 1 c. c. of normal sodium carbonate.[1]

Lead tubes were used in leading the air to and from the desiccator. The air entered it near the top and was withdrawn from near the bottom.

[1] For a criticism of volumetric methods of estimation of carbon dioxid, see Küster (15).

At the start of each experiment a current of carbon-dioxid-free air was led through the desiccator for about half an hour at the rate of from 1 to 2 liters per minute, this interval constituting a fore period during which the carbon dioxid was not collected. The absorber was then inserted in the train and the air passed through at the same rate as during the fore period. A water gauge inserted between the desiccator and the Reiset tube served to indicate the relative rate of flow. After an interval of from one to two hours the absorption apparatus was replaced by a freshly filled Reiset tube and the carbon dioxid collected for a second interval. The rate of respiration was thus determined during two successive intervals. In general, well-agreeing duplicates were obtained.

The figures showing the results of the study of the rate of respiration are given in Table VI. At the beginning the bananas were just on the point of turning yellow and were much more active (106 mg. of carbon dioxid per kg. hour) than the green fruit used in the calorimeter studies (30 mg. and 30 mg., respectively). The respiration was most intense (146 mg. per kg. hour) when the rate of transformation of starch was greatest. It then slowly slackened, reaching at the end 91 mg. per kg. hour.[a] As the rate of respiration of each sample analyzed was determined immediately before its analysis, the total amount of carbon dioxid evolved during the ripening period, 3.776 per cent of the original bananas, was easily estimated by a summation process from the data given in Table VI.

TABLE VI.—*Rate of respiration of detached bananas ripening in the humidity chamber*

Date.	Description.	Number of fruits.	Original weight.	Carbon dioxid collected.	Intervals for which collected.	Respiration rate.[b]	Average respiration rate.[b]	Interval from middle of one collection period to middle of next.	Average rate of respiration from middle of one period to middle of next.[b]
			Gm.	*Gm.*	*Hours.*	*Gm.*	*Gm.*	*Hours.*	*Gm.*
May 2	Wholly green, but on the point of turning yellow...	14	2,410	0.3344	1.33	0.104	0.106		
				.4576	1.75	.108		24	0.126
3	Bananas just beginning to turn yellow, sample ripening uniformly.....	15	2,552	.464	1.25	.145	.146		
				.656	1.75	.147		24	.142
4	Fruit yellower than on day previous, much green still present.....	14	2,506	.857	2.50	.136	.138		
				.669	1.92	.139		48	.128
6	Fully yellowed, but many specimens are green at the tips.....	13	2,300	.337	1.25	.117	.117		
				.675	2.53	.116		50	.113
8	Fully yellowed.....	16	2,835	.526	1.70	.109	.110		
				.352	1.12	.111		48	.108
10	Fruits beginning to brown slightly at the surfaces....	17	2,919	.453	1.47	.106	.106		
				.383	1.25	.105		70	.104
13	Skins brown, much yellow still present.....	14	[c] 2,520	.403	2.00	.101	.101		
16	Skins almost entirely brown, a little yellow present at ribs.....	14	[d] 2,535	.374	1.78	.091	.091	74	.096
				.376	1.78	.091			

[a] In the two calorimeter studies the respiration rate of the bananas reached maxima of 150 and 200 mg per kg. hour, respectively, and the respiratory activities at the end were 110 and 100 mg. per kg. hour, respectively.

[b] Grams carbon dioxid per kg. per hour.

[c] Weight used in calculating was 1,990 gm., three fruits having been rejected whose weight calculated to the original weight was subtracted from the original weight of the sample.

[d] Weight used in calculating was 2,310 gm., one fruit having been rejected, its weight calculated to original and subtracted.

The analytical data are given in Table V. The study of the detached fruit permitted a longer period of observation than when bananas attached to the stem were used. The rate of transformation of starch into soluble carbohydrates was very rapid at first—32.11 gm. per kg. per day. It then increased to 34.9 gm. per kg. per day. Starch hydrolysis, so far as revealed by analysis, nearly ceased six days before the end of the life history of the bananas. The analytical data confirm the facts developed in the earlier experiments.

DISCUSSION OF RESULTS

As the result of the foregoing studies, the author is in a position to state more exactly than has heretofore been possible the nature and extent of the changes in the composition of bananas during ripening. The most conspicuous change is the long-recognized conversion of starch into sugars. It is most rapid while the fruits are turning from green to yellow. During this period the respiration rate increases manyfold, becoming greatest at the time when the rate of starch hydrolysis is most rapid. Starch hydrolysis then gradually slackens, later ceasing altogether. The respiration rate, too, becomes slower, but still remains far more active than in the green fruit. Next to the starch and respiration changes, most conspicuous are those of water. The peel loses, while the pulp gains water steadily. The respective losses and gains in water of the peel and the pulp on ripening, expressed in terms of the original green bananas, are summarized in Table VII.

TABLE VII.—*Percentage of losses and gains in water of peel and pulp of bananas on ripening*

Experiment No.	Place of ripening.	Loss of water in peel.	Actual gain of water in pulp.	Gain of water in pulp corrected for water formed and absorbed in physiological processes.
1	Calorimeter	5.01	1.64	2.4
2	do	10.45	3.02	3.5
3	Humidity chamber	12.26	3.52	.....
4	do	11.59	3.94	.....
		12.12	4.51	.....
		11.64	4.63	.....
		14.23	6.34	6.135

In the first, second, and fourth experiments it is possible to show how much water is formed or absorbed by the pulp in physiological processes. The water formed in respiration can easily be calculated if formed in consequence of the complete combustion of carbohydrates and if the amount of carbon dioxid evolved on ripening in consequence of this combustion is known. The respiratory quotient and the thermal quotient determined by the Office of Nutrition Investigations for ripening bananas (16) agree in showing that the carbon dioxid evolved on normal ripening is due solely to the complete combustion of carbohydrates.

We are therefore justified in calculating the water formed by the equation $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$. From the water so formed is subtracted the water absorbed in the saccharification of starch. See Table VIII.

TABLE VIII.—*Percentage of water formed by the pulp of bananas in physiological processes*

Experiment No.	Carbon dioxid found.	Calculated water formed from equation I.	Loss of carbohydrate found on ripening in —[a]		Calculated water proportioned according to carbohydrate loss in—		Calculated water absorbed in pulp in starch hydrolysis.
			Peel.	Pulp.	Peel.	Pulp.	
1	1.334	0.546	0.46	0.88	0.188	0.358	1.14
2	2.256	.923	.41	1.43	.205	.718	1.23
4	3.776	1.545	.40	2.84	.190	1.355	1.16

[a] Probably slightly larger than actual on account of failure to completely estimate maltose. See p. 189.

In the first two experiments absorption of water amounting to 0.782 and 0.512 per cent occurred as a net result of respiration and starch hydrolysis. In the fourth experiment, where the bananas became overripe, the water formed in respiration was greater by 0.195 per cent than that absorbed in starch hydrolysis.

The increases of water in the pulp during ripening are all derived from the peel, except when bananas become overripe, when the water formed in respiration may more than balance the water absorbed in starch hydrolysis. From the quantity of sugar formed in the pulp it is evident that the osmotic pressure of the pulp must undergo a marked increase, with a corresponding decrease of vapor pressure, during the ripening of the fruit. A possible operating cause of the water transfer from peel to pulp is obvious.

From a knowledge of the carbon dioxid formed in respiration and knowing from the calorimeter data that carbon dioxid results from the complete combustion of carbohydrates, it can be determined whether or not the carbohydrates consumed in respiration were accurately made known from the analyses. Carbohydrate losses found by analysis contrasted with the expected losses from the calorimeter data are shown in Table IX.

TABLE IX.—*Comparison of carbohydrate losses with the expected losses from carbon dioxid in pulp of ripening bananas*

Carbohydrate losses found (expressed as hexose).	Expected losses from carbon dioxid formed in respiration.
Per cent.	*Per cent.*
1.34	0.91
1.84	1.54
3.24	2.58

By analysis somewhat greater losses appear than indicated from the calorimeter data. It is not improbable that the small differences are due to analytical error.

SUMMARY

(1) The usual carbohydrate changes—saccharification of starch, with formation of sucrose and invert sugar, and consumption of sugars in respiration—proceeded with uniformity in bananas of different bunches.

(2) The period of most rapid respiration corresponded closely with that of most rapid starch hydrolysis.

(3) The quantities of ash, protein, and ether extract underwent but slight changes during the ripening of the bananas. Pentosans decreased markedly in the pulp, but remained little changed in the peel.

(4) Analyses of the peel and pulp of ripening bananas showed a steady transfer of water from peel to pulp during ripening.

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ASSIMILATION OF COLLOIDAL IRON BY RICE

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INTRODUCTION

Previous work [1] at the Porto Rico Agricultural Experiment Station has shown that pineapples and upland rice grown on moderately or strongly calcareous soils are affected with chlorosis and that the failure of these plants to make a successful growth on such soils seems to be due wholly or in part to a diminished assimilation of iron. A determination of the forms of iron available to rice is therefore important. An experiment on the assimilation of colloidal iron is reported here, as it bears on this problem as well as on the properties of plant roots.

MATERIALS AND METHODS OF THE EXPERIMENT

The availability of colloidal iron was compared with that of ferric chlorid by growing upland rice in a nutrient solution. Water cultures were used in this work, as it is impossible to tell in what form the iron may be present in soil cultures. To prevent precipitation of the colloidal iron by other salts of the nutrient solution the iron was put in one flask (flask B) and the other salts in a second flask (flask A). The plants were grown with part of their roots in each flask.

The seeds were germinated over distilled water until they had developed two or more roots. Two plants were grown in each pair of flasks. Two hundred c. c. Erlenmeyer flasks of Jena glass with their necks joined together by surgeon's tape were used. The formula for the nutrient solution used in flasks A, which gave excellent results with rice in previous work, was as follows:

KNO_3	0.1017 gm.	$CaCl_2$	0.05 gm.
KH_2PO_4	.0714 gm.	$MgCl_2$	.05 gm.
$NaNO_3$	.2143 gm.	H_2SO_4	.5 c. c. N/10.
Na_2SO_4	.0315 gm.	Distilled water, 1,000 c. c.	

The plants were changed to fresh solutions every few days.

The colloidal iron used was the ordinary dialyzed iron. It contained 0.0383 gm. of Fe and 0.0058 gm. of Cl_2 per c. c. After salting out the colloidal iron with potassium sulphate, the filtrate was examined and found to contain the following, calculated as grams per c. c. of the original dialyzed iron: Cl_2, 0.00436 gm.; Fe, hardly a reaction with potassium

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sulphocyanate; acidity, equivalent to 0.002 gm. of Cl_2 from hydrochloric acid; ammonia, none with Nessler's reagent. The dialyzed-iron preparation after dilution was dialyzed for 24 hours with a parchment membrane without any iron appearing in the dialyzate.

While no soluble or ionized iron appeared in these tests we must assume that some existed in the dialyzed-iron preparation because of the high chlorin content. The nonappearance of iron in the dialyzate after dialysis and in the filtrate after salting out the iron was probably due to adsorption by the colloid or precipitate and to the strong hydrolysis that dilute ferric chlorid undergoes.[1] From the chlorin content it appears that about one-twelfth of the iron could have been present as ferric chlorid, but we can hardly assume that it was there as such or that a certain quantity of ferric chlorid in a colloidal-iron solution would act the same as in a simple aqueous solution. We can simply assume that the soluble iron bore some proportion to the quantity of chlorin. In the following tests, then, a low availability of the dialyzed-iron preparation is not proof of the assimilation of colloidal iron.

RESULTS OF EXPERIMENTS

EXPERIMENT I.—In a preliminary test the plants were grown for 42 days. The dialyzed iron was used at the rate of 10.5 parts of Fe per 100,000 parts of water for the first 10 days and at the rate of 1.05 parts of Fe per 100,000 for the remaining 32 days. The ferric chlorid was used at the rate of 0.41 part of Fe per 100,000. The results are given in Table I.

TABLE I.—*Growth of rice with dialyzed iron and ferric chlorid—Experiment I*

Nos. of flasks.	Solution in flasks A.	Solution in flasks B.	Green weight of tops.	Oven-dry weight of tops.	Gain over no-iron plants.
			Gm.	*Gm.*	*Gm.*
5–8	Nutrient solution without Fe.....	Distilled water.....	1.03	0.17	.....
9–12	.....do.....	Dialyzed iron.....	3.25	.54	0.37
13–16	.....do.....	Ferric chlorid.....	4.02	.67	.50
1–4	Nutrient solution + $FeCl_3$.....	Distilled water.....	5.82	.95	.78

The preparation of dialyzed iron had a certain availability, but relatively large amounts were less effective than the smaller quantity of ferric chlorid.

EXPERIMENT II.—In a second experiment the dialyzed iron and ferric chlorid were both used to furnish 0.4 gm. of Fe per 100,000 c. c. of water. The plants were grown for 59 days. The results are given in Table II.

[1] This was borne out by the following test: To 1 c. c. of dialyzed iron 0.00275 gm. of Fe from $FeCl_3$ was added; the solution was made to 100 c. c. and the colloidal iron salted out by K_2SO_4. The filtrate, tested for soluble iron (colorimetric method with KSCN), showed 0.00245 gm. of Fe had been lost. Of the 0.00245 gm. of Fe lost 0.00056 gm. was lost by adsorption by the precipitate. Precipitation by hydrolysis by water alone caused a loss of 0.00025 gm. of Fe, and hydrolysis in the presence of K_2SO_4 caused a loss of 0.00189 gm. of Fe.

TABLE II.—*Growth of rice with dialyzed iron and ferric chlorid—Experiment II*

Nos. of flasks.	Solution in flasks A.	Solution in flasks B.	Green weight of tops.	Oven-dry weight of tops.	Average oven-dry weight of tops.	Gain over no-iron plants.
			Gm.	*Gm.*	*Gm.*	*Gm.*
1-4	Nutrient solution without Fe.	Distilled water.....	3.38	0.60	0.60	.....
5-8	.....do.....	0.4 gm. of Fe per 100,000 c. c. from dialyzed iron.	4.41	.76	.....	.....
9-12	.....do.....	.....do.....	4.83	.79	.....	.....
12-16	.....do.....	.....do.....	4.71	.78	.78	0.18
17-20	.....do.....	0.4 gm. of Fe per 100,000 c. c. from $FeCl_3$.	6.40	1.07	.....	.....
21-24	.....do.....	.....do.....	5.61	1.05	.....	.....
25-28	.....do.....	.....do.....	7.82	1.31	1.14	.54

In this experiment, where equivalent and small quantities of iron were used, the dialyzed-iron preparation appeared to have an availability of about three-tenths that of the ferric chlorid.

EXPERIMENT III.—A third series was conducted, using equivalent small quantities of iron, ten times this amount of iron from dialyzed iron, and twice this quantity of iron from ferric chlorid. The results are given in Table III.

TABLE III.—*Growth of rice with dialyzed iron and ferric chlorid—Experiment III*

Nos. of flasks.	Solution in flasks A.	Solution in flasks B.	Green weight of tops.	Oven-dry weight of tops.	Average oven-dry weight of tops.	Gain over no-iron plants.	Average oven-dry weight of roots in flasks A.	Average oven-dry weight of roots in flasks B.
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1-5	Nutrient solution without Fe.	Distilled water.....	3.93	0.73	.....	.....	.....	.....
6-10	.....do.....	.....do.....	3.47	.69	0.71	.....	0.144	0.055
11-15	.....do.....	Fe from dialyzed iron, 0.4 gm. per 100,000 c.c.	4.35	1.06	.....	.....	.....	.....
16-20	.....do.....	.....do.....	3.75	.93	1.00	0.29	.201	.115
21-25	.....do.....	.....do.....	8.78	1.55	.....	.....	.....	.....
26-30	.....do.....	.....do.....	5.77	1.05	1.30	.59	.212	.194
31-35	.....do.....	Fe from $FeCl_3$, 0.4 gm. per 100,000 c. c.	5.51	1.00	.....	.....	.....	.....
36-40	.....do.....	.....do.....	6.69	1.24	1.12	.41	.217	.077
41-45	.....do.....	Fe from $FeCl_3$, 0.8 gm. per 100,000 c. c.	8.90	1.57	.....	.....	.....	.....
46-50	.....do.....	.....do.....	9.02	1.55	1.56	.85	.270	.174
51-55	Nutrient solution + $FeCl_3$.	Distilled water.....	17.92	2.81	.....	.....	.....	.....
56-60	.....do.....	.....do.....	20.03	3.13	2.97	2.26	.511	.190

Flasks Nos. 26 to 30 did not agree well with Nos. 21 to 25, so it is possible that the average of 1.30 gm. is too low. It is apparent from the preceding tests that the dialyzed iron was much less available than the ferric chlorid. On the basis of the Fe content, the dialyzed iron had to be present in at least five times the amount of ferric chlorid to produce the same yield.

The percentages of iron in the dry substance of the tops was determined in the plants from Experiment III. The percentages varied from

0.035 to 0.037 per cent of Fe_2O_3. Since the samples were small, it is felt that the figures merely show that practically the same percentages of iron were present in all the plants. Thus, the quantities of iron in the different lots of plants would vary as their dry weights.

In all the experiments the plants which received no iron in either flasks A or flasks B were strongly chlorotic, the chlorosis commencing about 6 to 10 days after the plants were put in the solutions. The plants receiving dialyzed iron or ferric chlorid in flasks B were also strongly chlorotic, although they were somewhat greener than the check plants without any iron, and the chlorosis was later in appearing. The plants which had the ferric chlorid added to the other nutrient salts in flasks A were of a normal green color.

The root development varied greatly in the different flasks. In all the flasks A which contained the complete nutrient solution without iron the root development was good, the main roots being long, with numerous long laterals. The roots in flasks B which contained only distilled water made very little growth and had few laterals. The roots in flasks B which contained only ferric chlorid made even less growth than those in distilled water. As soon as the roots penetrated the ferric-chlorid solution, the root tip appeared killed and no roots penetrated the solution for any distance. The roots in flasks B which contained dialyzed iron developed much better than in distilled water. During the latter stages of growth particularly a few heavy roots developed in the dialyzed iron, but these roots carried very few laterals. The oven-dry weights of the roots in flasks B given in Table III do not really give a comparison of the root developments in the solutions, for the reason that the weights of the roots in flasks B, Nos. 31–60, were made up chiefly of root "stubs"—heavy roots which started out from the plants but did not develop in the distilled water or ferric-chlorid solution. The larger the top growth, dependent on the amount of iron obtained, the more root "stubs" developed. For instance, flasks B, Nos. 41–46 and Nos. 36–50, had 0.174 gm. of roots, while Nos. 31–35 and Nos. 36–40 had but 0.077 gm. of roots. As a matter of fact, the root development in the solution was less in the first case than in the second.

DISCUSSION OF RESULTS

The poor development of the roots in the flasks B was, of course, due to the well-established toxicity of unbalanced solutions—in this case of single salts or distilled water.[1] Because of the injury resulting from the iron solutions it is impossible to draw a very sharp conclusion concerning the assimilation of colloidal iron. Although the roots developed better in

[1] True holds the injury from ordinary distilled water "but a special case of the general type of injury wrought on cells by unbalanced solutions."—True, R. H. The harmful action of distilled water. *In* Science, n. s., v. 39, no. 999, p. 295. 1914.

the dialyzed iron than in the ferric chlorid,[1] they assimilated less iron, even in solutions containing three to five times more dialyzed iron than ferric chlorid. It seems probable that the small amount of iron obtained from the dialyzed iron was not colloidal iron but soluble iron. It is true that the tests made of the dialyzed-iron preparation revealed little or no ionized iron, but we must assume the presence of some soluble iron from the chlorin content of the preparation.

In view of the low availability of the dialyzed-iron solution it would seem that the often-mentioned but questionable acid excretion of roots was not operative, at least not in this unbalanced solution. With respect to fineness of division and contact with the roots the colloidal iron was especially favorable for assimilation by an acid if the roots had excreted such. An apparent objection to the conclusion that colloidal iron is not assimilable is the fact that dialyzed iron is sometimes used in nutrient solutions and that in Von Crone's nutrient solution ferric phosphate is employed. In such solutions, however, it is not at all certain that the plants utilize insoluble iron compounds. In fact, from further work in progress it appears that rice at least is capable of assimilating only soluble iron in nutrient solutions.

Aside from the question of the assimilation of colloidal iron, the preceding test is of interest in connection with the study of unbalanced solutions. The fact that while some roots of the plant were developing well in the balanced solution of the flasks A other roots of the same plant were injured by the distilled water or ferric chlorid of the flasks B shows that in case the toxicity of certain salt solutions or ordinary distilled water is due to injury in the root cells by extraction of other electrolytes,[2] this extraction takes place faster than the electrolytes can be supplied from other parts of the plant. For in this case the roots in flasks A had an abundance of the electrolytes to draw on.

The idea that the toxicity of single-salt solutions is due to or accompanied by the penetration of the salts finds confirmation in the preceding experiments. It is evident from Experiment III that the stronger the ferric chlorid solution the less the root development in the solution, but the greater the amount of iron absorbed (as shown by the top growth which was dependent on iron absorbed). Osterhout[3] has shown by electrical conductivity measurement that single-salt solutions penetrate cells of the kelp, Laminaria.

[1] The reduction of the toxicity of distilled water by colloids and finely divided solids has been frequently noted.

[2] True, R. H. Loc. cit.

[3] Osterhout, W. J. V. The permeability of protoplasm to ions and the theory of antagonisms. *In* Science, n. s., v. 35, no. 890, p. 112–115, 1912.

SUMMARY

The work reported would seem to show that rice can not assimilate colloidal iron. It is believed that the iron obtained from the dialyzed-iron preparation was soluble iron.

It is apparent that the toxicity of ordinary distilled water or ferric-chlorid solutions for plant roots can not be overcome by supplying other roots of the same plant with a balanced solution.

The toxicity of the ferric-chlorid solution was accompanied by the penetration of iron into the root and transportation to the leaves.

COLORING MATTER OF RAW AND COOKED SALTED MEATS

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INTRODUCTION

The red color of fresh lean meat, such as beef, pork, and mutton, is due to the presence of oxyhemoglobin, a part of which is one of the constituents of the blood remaining in the tissues, while the remainder is a normal constituent of the muscles. When fresh meat is cooked or is cured by sodium chlorid, the red color changes to brown, owing to the breaking down of the oxyhemoglobin into the two constituents, hematin, the coloring group, and the proteid, globin.

On the other hand, when fresh meat is cured by means of a mixture of sodium chlorid and a small proportion of potassium nitrate, or saltpeter, either as a dry mixture or in the form of a pickle, the red color of the fresh meat is not destroyed during the curing process, the finished product having practically the same color as the fresh meat. Neither is the red color destroyed on cooking, but rather is intensified.

The practical value of saltpeter in the curing of meats is so well known that its use for this purpose may be said to have become practically universal; such use is not confined to the commercial meat-packing industry, but it is used in the home curing of meats as well.

It is only within comparatively recent years, however, that anything very definite has been known concerning the nature of the color of salted meats or the process of the color formation. The work which is reported in this paper was undertaken for the purpose of obtaining more complete information concerning the color of raw and cooked salted meats.

HISTORICAL SUMMARY

Weller and Riegel (1897),[1] in the examination of a number of samples of American sausages, obtained a red coloring matter on extracting the samples with alcohol and other solvents, which color they concluded to be in some manner due to the action of the salts used in curing upon the natural color of the meat. On account of similarity of spectra, this color was considered to be methemoglobin.

Lehmann (1899) observed that when fresh meat was boiled in water containing nitrites and free acid or in old meat broth the surface of the meat turned bright red in color, in contrast to the brown color which fresh meat takes on when boiled in water free from nitrites. The addi-

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 225.

tion of nitrates to the water did not cause the production of the red color on the surface of the meat on boiling. This color was found to be soluble in alcohol and ether and to give a spectrum showing an absorption band just at the right of the D line, and a second band, often poorly defined, at the left of the E line. On standing, the color of the solution changed to brown and gave the spectrum of alkaline hematin.

A color with similar properties was obtained on extracting hams and various kinds of sausages with alcohol and other solvents. The author named this coloring matter "hæmorrhodin."

Kisskalt (1899) studied the production of red color in fresh meats on cooking and found that this color appeared when meat was cooked in bouillon which was several days old, or in water containing nitrites and free acid. Meat boiled in water to which saltpeter had been added did not take on the red color; but, on the other hand, if the meat was first allowed to stand several days in contact with saltpeter and then boiled, the red color appeared.

Haldane (1901) made an extensive study of the color of cooked salted meat, which color he concluded to be due to the presence of the nitric-oxid hemochromogen resulting from the reduction of the coloring matter of the uncooked meat, or nitric-oxid hemoglobin (NO-hemoglobin). This color was found to be the same as that resulting from the boiling of fresh meat in water containing nitrites and free acid. It exhibited a spectrum showing a distinct band just at the right of the D line and a faint band a trifle to the left of the E line. The color was found to be soluble in alcohol and in ether and to be quite resistant to the action of reducing agents.

The color of uncooked salted meats was found to be soluble in water and gave a spectrum characteristic of NO-hemoglobin. The formation of the red color in uncooked salted meats is explained by the action of nitrites in the presence of a reducing agent and in the absence of oxygen upon hemoglobin, the normal coloring matter of fresh meats.

Orlow (1903) states that the red color of sausages is due to the action upon the color of the fresh meat of the nitrites resulting from the reduction of the saltpeter used in the process of manufacture.

The present author (1908) studied the action of saltpeter upon the color of meat and found that the value of this agent in the curing of meats depends upon its reduction to nitrites and nitric oxid, with the consequent production of NO-hemoglobin, to which compound the red color of salted meats is due. Saltpeter, as such, was found to have no value as a flesh-color preservative.

Glage (1909) is the author of a pamphlet concerning practical methods for obtaining the best results from the use of saltpeter in the curing of meats and in the manufacture of sausages. The fact that the value of saltpeter as a flesh-color preservative is dependent upon the reduction of the nitrate to nitrite is recognized, and directions are given for the

partial reduction of the saltpeter to nitrites by heating the dry salt in a kettle before it is to be used. It is stated that this partially reduced saltpeter is much more efficient in the production of color in the manufacture of sausage than is the untreated saltpeter.

Humphrey Davy in 1812 (cited by Hermann, 1865) and Hoppe-Seyler (1864) noted the action of nitric oxid upon hemoglobin, but it appears that Hermann (1865) was the first to furnish us with much information as to the properties of this derivative of hemoglobin. He prepared NO-hemoglobin by first passing hydrogen through dog's blood until spectroscopic examination showed that all of the oxyhemoglobin had been reduced to hemoglobin, then saturating the blood with pure nitric oxid prepared from copper and nitric acid, and finally again passing hydrogen through the blood to remove all traces of free nitric oxid. It was observed that the spectrum of hemoglobin had changed to one showing two bands in practically the same position as those of oxyhemoglobin. The blood saturated with nitric oxid was found to be darker in color than either arterial blood or that saturated with carbon monoxid, and on exposure to air or on treatment with ammonium sulphid it proved to be as stable as carbon-monoxid hemoglobin.

NO-hemoglobin is mentioned but briefly in most of the recent texts on physiological or organic chemistry as being a hemoglobin derivative of but little practical importance. Abderhalden (1911) and Cohnheim (1911), however, describe this compound quite fully.

EXPERIMENTS WITH NO-HEMOGLOBIN

The following studies on NO-hemoglobin were conducted by the writer.

Formation.—Nitric oxid was prepared by treating copper foil with concentrated nitric acid, and the production of the gas was carried on long enough to free the apparatus from higher oxids of nitrogen before conducting gas into the solution of hemoglobin. A simple apparatus was arranged so that the sample of blood or of hemoglobin to be treated was first saturated with pure hydrogen, then with nitric oxid, and finally again with hydrogen to remove all free nitric oxid. Great care was exercised to exclude air from the apparatus, since the presence of even a small amount of free oxygen results in the oxidation of nitric oxid to nitrogen peroxid, with the consequent production of nitrous and nitric acids, which act upon hemoglobin to form methemoglobin.

NO-hemoglobin was prepared, in the manner described above, both from defibrinated blood and from a solution of oxyhemoglobin prepared by Hoppe-Seyler's method. It was found, as a rule, that if a solution of oxyhemoglobin or of defibrinated blood was treated with nitric oxid, with all precautions to exclude free oxygen, the NO-hemoglobin usually contained a small amount of methemoglobin. This fact has been noticed by other investigators and is due to the union of the loosely

bound oxygen of the oxyhemoglobin with the nitric oxid to form nitrogen peroxid, which, as has just been noted, acts upon the hemoglobin to form methemoglobin.

Some of the earlier investigators have found that small quantities of oxyhemoglobin could be reduced to hemoglobin by means of hydrogen; but it has been the experience of the writer that, when working with any considerable quantity of oxyhemoglobin, practically no reduction took place even after passing a current of hydrogen through an oxyhemoglobin solution for several hours. In practice it was found best to reduce oxyhemoglobin to hemoglobin by means of hydrazin hydrate before saturation with nitric oxid.

PROPERTIES.—NO-hemoglobin in concentrated solution has a dark cherry-red color; in dilute solutions it has a light cherry-red color, in contrast to the bright-red color of oxyhemoglobin or to the purple-red color of hemoglobin in solutions of the same concentration. In solutions free from methemoglobin NO-hemoglobin is quite stable, and solutions of the compound have been kept in a refrigerated room at 32° to 35° F. for several weeks without apparent change. On boiling a solution of NO-hemoglobin a brick-red precipitate is formed, in contrast to the dark-brown precipitate formed on boiling a solution of oxyhemoglobin or hemoglobin.

NO-hemoglobin shows a characteristic spectrum consisting of a heavy band just at the right of the D line and a somewhat lighter and wider band a trifle to the left of the E line (fig. 1). These absorption bands occupy practically the same positions as those of oxyhemoglobin, but are distinguishable from the latter in solutions of the same concentration by lower intensity, less sharply defined edges, and by the fact that when a solution of oxyhemoglobin is treated with a reducing agent—e. g., hydrazin hydrate—the characteristic single broad band of hemoglobin appears, while on treating a solution of NO-hemoglobin with the same reagent, no reduction takes place and the bands are not affected.

NO-hemoglobin is practically unaffected on treatment with potassium ferricyanid in neutral solution, with Stokes's solution (ammoniacal ferro-tartrate), with sodium nitrite, or with hydrazin hydrate, but is gradually reduced by potassium ferricyanid in acid solution.

When a solution of NO-hemoglobin is treated with ether in the presence of a small quantity of alcohol or of dilute acid, a bright-red colored extract is obtained which shows a distinct absorption band just at the right of the D line, and occasionally, in concentrated solution, a faint band at the left of the E line. In the absence of acid or alcohol no color is extracted by ether. In a previous paper the writer considered that the color extracted under the above conditions was NO-hemoglobin, but from work which he has done since that time it is evident that the color extracted by ether is a derivative of NO-hemoglobin, produced apparently by the reducing action of the alcohol or acid upon the NO-

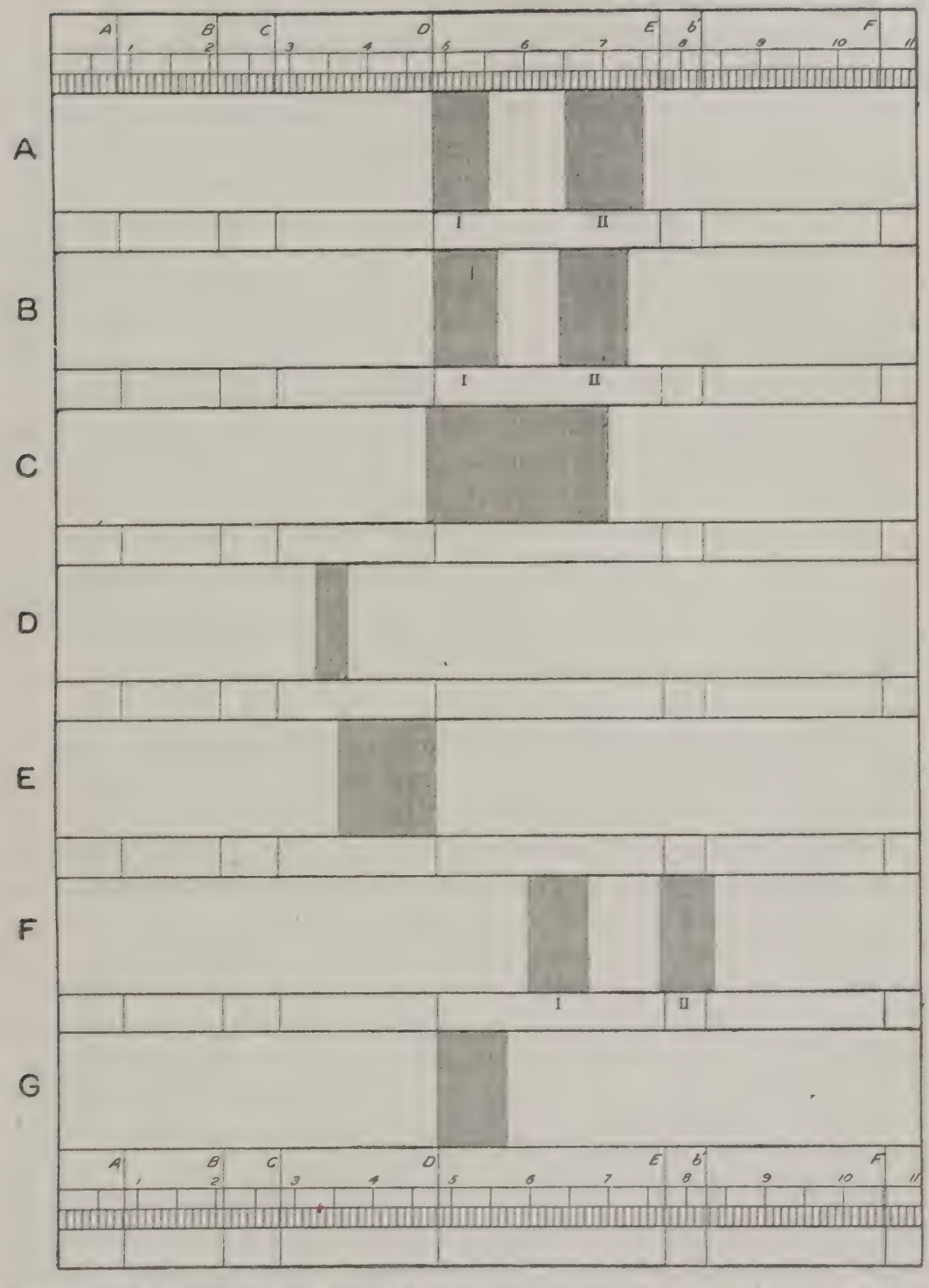

FIG. 1.—Spectra of hemoglobin and some of its derivatives: *A*, absorption spectrum of a solution of oxyhemoglobin; *B*, absorption spectrum of a solution of NO-hemoglobin; C, absorption spectrum of a solution of hemoglobin prepared by treating a solution of oxyhemoglobin with hydrazin hydrate; *D*, absorption spectrum of a solution of methemoglobin prepared by treating a solution of oxyhemoglobin with potassium ferricyanid; *E*, absorption spectrum of an alkaline solution of hematin; *F*, absorption spectrum of a solution of hemochromogen prepared by treating an alkaline solution of hematin with hydrazin hydrate, *G*, absorption spectrum of a solution of NO-hemochromogen.

hemoglobin. In pure solutions nitric oxid is insoluble in ether. The nature of this ether-soluble derivative of NO-hemoglobin will be discussed in connection with the color of cooked salted meats.

PREPARATION OF PURE NO-HEMOGLOBIN IN DRY CONDITION.—Twenty-five c. c. of a concentrated solution of NO-hemoglobin were cooled to 0° C., 6 c. c. of absolute alcohol previously cooled to the same temperature were added, and the dish was gently rotated. An abundant quantity of dark cherry-red crystals formed immediately. The dish was covered, placed in a refrigerated compartment for 24 hours at a temperature of −4° C., and the contents then filtered with the aid of suction in a room held at a temperature of +1° to +4° C. There was obtained a quantity of reddish brown crystals which were partly soluble in water, giving a reddish brown solution which showed a spectrum of NO-hemoglobin contaminated by the presence of methemoglobin. The material was not sufficiently soluble in water to allow of recrystallization.

A large number of trials were made with various methods of procedure in an endeavor to obtain pure NO-hemoglobin in dry condition, but without much success. Crystallization by a method using alcohol seems to change the NO-hemoglobin, in part at least, to methemoglobin.

When crystallization was carried on in the presence of a reducing agent—e. g., hydrazin hydrate or Stokes's solution—moist crystals could be obtained which showed a spectrum of NO-hemoglobin free from methemoglobin; but on drying the crystals in vacuo over sulphuric acid a change to methemoglobin took place.

CRYSTALLIZATION OF NO-HEMOGLOBIN.—In general, the method used by Reichert and Brown (1909) for the crystallization of hemoglobin and oxyhemoglobin was followed. In brief, the procedure was as follows: A pure concentrated solution of NO-hemoglobin was prepared by the methods previously described, and was examined spectroscopically to determine its freedom from other hemoglobin derivatives. Ammonium oxalate, in the proportion of 2 gm. to 100 c. c., was then added to the solution, which was then shaken to dissolve the salt. All subsequent procedure was carried on in a refrigerated room at a temperature of +1° to +5° C.

A few drops of the NO-hemoglobin solution were placed on a microscopic slide and allowed to evaporate until a heavy, dry proteid ring had formed around the drop. The cover glass was then carefully applied so as to exclude air bubbles, and the edges were sealed with balsam. Microscopic examination was made immediately after mounting and at intervals thereafter, according to the rate of crystal formation. The formation of crystals started at the dried proteid ring and proceeded toward the center of the mount, although occasionally crystals of ammonium oxalate would form, and the hemoglobin crystals would start from this base.

The above method was followed for the crystallization of both oxyhemoglobin and NO-hemoglobin from sheep, ox, and pig blood, and of methemoglobin from ox blood. It was found practically impossible to secure crystals of hemoglobin or of its derivatives from the blood of the above-mentioned animals without the use of ammonium oxalate. It was also found necessary to carry on the work at a temperature slightly above freezing, since the crystals would not form readily at room temperature.

OX-BLOOD OXYHEMOGLOBIN.—Plate XXXII, figures 1 and 2, shows crystals of oxyhemoglobin from ox blood. These crystals correspond very closely with those described by Reichert and Brown. No attempt was made to make a critical study of the crystallography of any of the various hemoglobin compounds studied.

OX-BLOOD NO-HEMOGLOBIN.—Plate XXXII, figure 3, shows crystals of this compound. It was found very difficult to obtain crystals of NO-hemoglobin, owing, apparently, to its greater solubility as compared with oxyhemoglobin. The crystalline structure, it will be noted, is distinctly different from that of oxyhemoglobin.

OX-BLOOD METHEMOGLOBIN.—Plate XXXII, figures 4 and 5, shows crystals of this compound which were prepared by treating oxyhemoglobin with potassium ferricyanid. It will be noted that the crystals of this compound are very similar in structure to those of NO-hemoglobin.

SHEEP-BLOOD OXYHEMOGLOBIN.—Plate XXXIII, figures 1 and 2, shows crystals of this compound which correspond very closely with those described by Reichert and Brown.

SHEEP-BLOOD NO-HEMOGLOBIN.—It was not found possible to secure a thoroughly satisfactory mount of these crystals, owing to their high solubility. Plate XXXIII, figure 3, shows fair crystals of this compound in the form of plates, which, however, are largely obscured by the large crystals of ammonium oxalate. It may be noted, however, that the crystals of NO-hemoglobin are distinctly different from those of oxyhemoglobin from the same source.

PIG-BLOOD NO-HEMOGLOBIN.—Plate XXXIII, figures 4 and 5, shows crystals of NO-hemoglobin obtained from pig's blood. It was not found possible to secure a good mount of oxyhemoglobin crystals from pig's blood, but the NO-hemoglobin crystals shown in this illustration are very different in structure from the oxyhemoglobin crystals from pig's blood described by Reichert and Brown.

The above work shows that NO-hemoglobin derived from ox, sheep, and pig blood is a crystallizable compound, with a definite structure, depending upon the species from which it has been obtained, and that its structure is different from oxyhemoglobin crystals from the same sources.

COLOR OF UNCOOKED SALTED MEATS

To a sample of finely ground fresh beef was added 0.2 per cent of potassium nitrate, and the material was placed in a refrigerated room at a temperature of 34° F. for seven days. At the end of that period the meat had a bright-red color, but gave evidences of incipient putrefaction. On extraction with 95 per cent alcohol, a faintly red-colored extract was obtained, which on spectroscopic examination showed a faint band just at the right of the D line. The color of the extract quickly faded. On extraction with water the sample yielded a dark-red extract, which retained its color without change for several days. On spectroscopic examination the following spectrum was observed: Just at the right of the D line a very heavy dark band, and a trifle to the left of the E line a heavy band, but wider than and not quite so dark as the first band and with less sharply defined edges. These bands correspond exactly in position with those of oxyhemoglobin and NO-hemoglobin, but more closely resemble the latter.

On treatment with sodium-nitrite solution the extract changed somewhat in color to a reddish brown, but still showed the two absorption bands noted above, and in addition showed a methemoglobin band at the left of the D line. The presence of this band would indicate that the extract contained some oxyhemoglobin, which on treatment with sodium nitrite changed to methemoglobin. On treatment with hydrazin hydrate the extract was not changed in color, and showed the following spectrum: Just at the right of the D line a very heavy band; at the left of the E line a somewhat lighter and wider band with less sharply defined edges. This spectrum corresponds exactly with that of the original extract.

The position of the absorption bands just noted, the fact that these bands were unaffected on treatment of the extract with hydrazin hydrate, and the solubility of the color in water are sufficient proof that the color of the meat under examination was due to the presence of NO-hemoglobin.

The production of NO-hemoglobin in the sample of meat under examination is easily explained. The potassium nitrate added to the meat had been reduced, either by bacterial or enzym action or by both, to potassium nitrite, nitrous acid, and nitric oxid, with the consequent formation of NO-hemoglobin.

Samples of various uncooked meats and sausages in which saltpeter had been used as one of the curative agents were obtained on the market for the purpose of studying the coloring matter of these products, and the following results were obtained:

SMOKED PORK SHOULDER.—The freshly cut surface of the meat was bright red in color. On extraction with water the finely ground lean meat gave a dull-red colored extract, which showed the following spectrum: Just at the right of the D line a fairly heavy dark band. No other

bands were visible. On treatment with potassium ferricyanid the red color of the solution changed to brown and the band disappeared. On extraction with 95 per cent alcohol a red-colored extract was obtained which showed the following spectrum: Just at the right of the D line a fairly heavy band, in the same position as noted with the water extract. On treatment with potassium ferricyanid the color of the extract changed to brown, and the absorption band disappeared. The addition of sodium nitrite or of hydrazin chlorid caused practically no change in the color or spectrum of the extract.

SMOKED PORK HAM.—The cut surface had a bright-red color. On extraction of the finely-ground lean meat with water a red-colored extract was obtained which showed the following spectrum: A fairly heavy band just at the right of the D line. The addition of sodium nitrite or of hydrazin hydrochlorid caused no change in color of the extract.

SALAMI SAUSAGE.—This product is known as a dry summer sausage, and is cured by hanging in a dry and well ventilated room for some time; it is also subjected to a light smoking. The cut surface of the meat had a bright-red color. On extraction with alcohol a bright-red colored extract was obtained which showed the following spectrum: A heavy dark band just at the right of the D line. On treatment with hydrazin chlorid the solution became somewhat milky in appearance, but the color and spectrum were not affected. Treatment with sodium nitrite caused no change in the color or spectrum of the extract, while potassium ferricyanid changed the red color of the solution to brown, and the absorption band disappeared.

DRIED BEEF.—This product is first cured in a brine containing salt, sugar, and saltpeter, and is then smoked. The cut surface of the meat was dark red. On extraction with alcohol a bright-red colored extract was obtained, which showed the following spectrum: A heavy dark band just at the right of the D line. Treatment with reducing agents gave the following results: Potassium ferricyanid destroyed the red color, and the absorption band practically disappeared; sodium nitrite partially destroyed the red color, but the absorption band at the right of the D line was still visible; hydrazin hydrate did not affect the color or spectrum of the extract. On extraction with water a faintly red-colored solution was obtained. The meat residue after extraction had a bright-red color.

SMOKED CERVELAT SAUSAGE.—This product is prepared in much the same manner as salami sausage. On extraction with alcohol a light-red colored extract was obtained which gave an absorption spectrum showing a distinct band just at the right of the D line. Treatment with hydrazin hydrate did not affect the color or spectrum of the solution, while sodium nitrite, hydrazin chlorid, and potassium ferricyanid destroyed the red color of the extract, and the absorption band disappeared.

Treatment of the sausage with water extracted no color, while ether gave a light-red colored extract, the color of which faded rapidly. Spectroscopic examination showed a distinct band just at the right of the D line.

CURED PORK SHOULDER, NOT SMOKED.—The freshly cut surface of the meat had a bright-red color. On treatment with water a light-red colored extract was obtained which showed the following spectrum: A distinct band just at the right of the D line. The color of the extract faded rapidly. On extraction with alcohol the meat gave a red-colored extract, showing a spectrum with a distinct band just at the right of the D line. Treatment with sodium nitrite or with hydrazin hydrate caused practically no change in the color or spectrum of the extract.

CORNED BEEF.—On extraction with water a rather cloudy, red-colored extract was obtained which gave a spectrum showing a fairly distinct band just at the right of the D line. Treatment with sodium nitrite caused no change in the color or spectrum of the solution. On extraction with alcohol the meat gave a bright-red colored extract which showed a fairly heavy absorption band just at the right of the D line. Treatment of the extract with sodium nitrite did not affect the color or spectrum, while hydrazin hydrate, hydrazin chlorid, and potassium ferricyanid destroyed the red color and the absorption bands of the solution.

SUMMARY OF RESULTS WITH UNCOOKED SALTED MEATS

The results of the examination of the samples of uncooked salted meats reported above may be summarized as follows:

The color of the meats was generally soluble in alcohol and in some cases in water. All samples gave extracts which showed an absorption band just at the right of the D line. In general, treatment with hydrazin hydrate or with sodium nitrite did not affect the color or spectrum of the extract. Potassium ferricyanid and hydrazin chlorid generally destroyed the red color of the extract and caused the absorption band to disappear. The fact that all samples of meats examined gave a red-colored extract with alcohol while only a part of them yielded a red-colored extract with water may be explained in this way: NO-hemoglobin is readily soluble in water, but insoluble in alcohol or ether. However, if a little alcohol is first added to a solution of NO-hemoglobin—sufficient to cause a slight precipitation of the proteid—and the solution is then extracted with ether, a bright-red colored extract may be obtained which gives a spectrum showing an absorption band just at the right of the D line. The coloring matter extracted by ether is undoubtedly a decomposition product of NO-hemoglobin.

In the case of the meats examined it would appear that all samples giving with water a red-colored extract that showed an absorption band

at the right of the D line had as their coloring matter NO-hemoglobin. In the case of those samples giving a red-colored extract with alcohol and showing an absorption band at the right of the D line but giving no red-colored extract with water, the coloring matter of the meat was not NO-hemoglobin, but a derivative of that compound. In the case of those samples which gave red-colored extracts with both alcohol and water and which showed an absorption band just at the right of the D line, the color is certainly due, in part at least, to NO-hemoglobin, and it may be due, in part, to a derivative of NO-hemoglobin present as such in the meat, or the alcohol may break down the NO-hemoglobin during extraction.

The evidence is ample to show that the action of saltpeter in the curing of meats is primarily to cause the formation of NO-hemoglobin; but it is very possible that under certain conditions of manufacture or processing to which salted meats are subject, the NO-hemoglobin may undergo changes.

RED COLOR OF COOKED SALTED MEATS

Haldane has shown that the red color of cooked salted meats is due to the presence of NO-hemochromogen, a reduction product of NO-hemoglobin to which the color of uncooked salted meats is due. NO-hemochromogen is characterized by its solubility in alcohol, its resistance to the action of reducing agents, and by a spectrum showing a distinct band just at the right of the D line and a faint band a trifle to the left of the E line. While Haldane's work seems to show clearly that the color of cooked salted meats is due to NO-hemochromogen, it has seemed desirable to study the subject further and to determine especially if the NO-hemoglobin of uncooked meats be reduced to NO-hemochromogen under other conditions than by cooking. The fact that in the examination of certain uncooked salted meats a coloring matter had been obtained similar to NO-hemoglobin yet not possessing all of the properties of that compound, as has already been noted, led the writer to believe that the coloring matter of some uncooked salted meats might be due, in part at least, to NO-hemochromogen.

NO-hemochromogen is but briefly mentioned in the literature. The compound is described by Linossier (1887), Haldane (1901), and by Abderhalden (1911).

The following experiments in the production of this compound were conducted by the writer. A dilute alcoholic, ammoniacal solution of hematin was saturated, first with hydrogen to remove air, then with nitric oxid, and finally again with hydrogen. On treatment with nitric oxid the brown color of the solution gradually changed to reddish brown; the characteristic spectrum of alkaline hematin disappeared, and in its place appeared a spectrum showing a fairly heavy band just at the right

of the D line and corresponding to the spectrum of NO-hemochromogen, as described by Haldane, except that the second band at the left of the E line was not visible. The addition of Stokes's solution caused no change in the color or spectrum of the solution. On standing overnight the red color of the solution had changed to brown and the spectrum had changed to that of alkaline hematin.

A dilute alcoholic, ammoniacal solution of hematin was treated with a small quantity of a concentrated solution of hydrazin sulphate. The brown color of the hematin solution quickly changed to the bright pink of hemochromogen, with a corresponding change in spectrum. The hemochromogen solution was then saturated with hydrogen, nitric oxid, and hydrogen in turn. On saturation with nitric oxid the pink color of the solution quickly changed to cherry red, and spectroscopic examination showed a heavy dark band just at the right of the D line. No other bands were observed, even though the solution was examined in a deep absorption cell.

The two substances produced, either by saturation of a solution of hematin with nitric oxid or by similar treatment of a solution of hemochromogen, are apparently identical. The evidence seems to show that this substance is the compound NO-hemochromogen.

The structural relation between NO-hemoglobin and NO-hemochromogen is simple. NO-hemoglobin is a molecular combination of nitric oxid and hemoglobin—the latter compound consisting of the proteid group, globin, on one hand, and the coloring group, hemochromogen, on the other. NO-hemoglobin and NO-hemochromogen differ from each other simply in that one contains the proteid group, globin, while the other does not. Apparently, then, a method of treatment which would split off the globin group from NO-hemoglobin should result in the production of NO-hemochromogen, provided, of course, that the procedure did not in turn change or destroy the NO-hemochromogen produced.

As has already been noted by Haldane, it was found that when a solution of NO-hemoglobin was heated to boiling a brick-red precipitate formed, in contrast to the dark-brown precipitate which formed on heating a solution of oxyhemoglobin or of blood. The brick-red precipitate was filtered off and was then extracted with alcohol, which gave a light-red colored extract showing a spectrum with a fairly heavy band just at the right of the D line. This spectrum corresponds with that of NO-hemochromogen. On standing, the color of the extract faded rapidly.

A solution of oxyhemoglobin was treated with one-hundredth normal nitrous acid and showed a spectrum corresponding to that of NO-hemoglobin. The color of the solution changed from the bright red of oxyhemoglobin to the dark cherry-red characteristic of NO-hemoglobin. Treatment with sodium nitrite did not affect the color or spectrum of the

solution. The production of NO-hemoglobin by this method is undoubtedly due to the action of the nitric oxid, liberated from the unstable nitrous acid, upon the hemoglobin. On heating the solution to boiling a brick-red precipitate was formed which, after filtration and on extraction with alcohol, gave a light-red colored extract which showed a spectrum with a fairly heavy band just at the right of the D line. Treatment with sodium nitrite did not affect the color or spectrum of the solution.

A piece of lean beef about 3 inches square and 1¼ inches thick was placed in a cold 0.1 per cent solution of sodium nitrite, and the solution was brought to the boiling point and boiling continued for one-half hour. Immediately on placing in the cold nitrite solution the surface of the meat turned dark brown in color; but on boiling, the meat took on a bright-red color, similar to that of cooked corned beef. On cutting the cooked meat the cut surface showed a bright-red color extending from the surface for about one-fourth of an inch toward the center of the piece, the interior of the meat being dark brown. The meat was ground and extracted with alcohol, which gave a bright-red colored extract showing a spectrum with a quite heavy band at the right of the D line and a very faint band at the left of the E line.

The color which Lehmann obtained on extracting with alcohol meat which had been cooked in water containing nitrites and acid, he named "hæmorrhodin." Results obtained by Haldane and by the writer seem to show, however, that the compound described by Lehmann is none other than NO-hemochromogen.

The following kinds of salted meats were used in studying the color of cooked salted meats: Salami, cervelat, Frankfurter, and Bologna sausages, corned beef, cured pork shoulder (unsmoked), smoked shoulder, and smoked ham. Bologna and Frankfurter sausages are cooked in the process of manufacture, so that these products are cooked salted meats. Portions of each of the samples of meats were cooked for some time in boiling water, then finely ground and extracted with alcohol. In all cases a red-colored extract was obtained, the intensity of the color varying with the product. On spectroscopic examination the extract from each sample showed a distinct band just at the right of the D line, but in practically all cases a second absorption band was not visible. The addition of sodium nitrite, as a rule, did not affect the color or spectrum of the extract. The color of the cooked salted meats was soluble in ether, but insoluble in water.

The evidence seems to show very clearly that the color of cooked salted meats is due to the NO-hemochromogen resulting from the reduction of the NO-hemoglobin of the raw salted meats on boiling.

It was found that the alcoholic extracts from the same product, whether in raw or cooked condition, showed practically identical prop-

erties. This does not signify that the coloring matter of raw salted meat is necessarily NO-hemochromogen, but rather it appears that treatment with alcohol reduces the coloring matter of uncooked salted meats, NO-hemoglobin, to NO-hemochromogen. In the case of certain uncooked salted meats NO-hemochromogen may be present as such before extraction with alcohol. The best means of differentiating between NO-hemoglobin and NO-hemochromogen as the coloring matters of salted meats seems to be on the basis of their differences in solubility. NO-hemoglobin is soluble in water but insoluble in ether and alcohol, while NO-hemochromogen is soluble in ether and alcohol and insoluble in water. In other respects the properties of the two compounds are very similar.

In the case of certain kinds of uncooked salted meats and meat food products—e. g., summer sausage and dried beef—the coloring matter seems to be NO-hemochromogen rather than NO-hemoglobin. The color is insoluble in water, but is soluble in alcohol and exhibits other properties of NO-hemochromogen.

It is very probable that in the case of meats which have been cured with saltpeter or of meat food products in which saltpeter has been used in the process of manufacture, the reduction of NO-hemoglobin to NO-hemochromogen takes place to a greater or lesser degree, depending upon conditions of manufacture and storage. The two compounds are so closely allied that their differentiation in one and the same product is not a matter of great importance.

CONCLUSIONS

The results of the investigation reported in this paper may be briefly summarized as follows:

The color of uncooked salted meats cured with potassium nitrate, or saltpeter, is generally due, in large part at least, to the presence of NO-hemoglobin, although the color of certain kinds of such meats may be due in part or in whole to NO-hemochromogen.

The NO-hemoglobin is produced by the action of the nitric oxid resulting from the reduction of the saltpeter used in salting upon the hemoglobin of the meat.

The color of cooked salted meats cured with saltpeter is due to the presence of NO-hemochromogen resulting from the reduction of the color of the raw salted meat on cooking.

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PLATE XXXII

Fig. 1.—Oxyhemoglobin, ox blood.

Fig. 2.—Oxyhemoglobin, ox blood.

Fig. 3.—NO-hemoglobin, ox blood.

Fig. 4.—Methemoglobin, ox blood. Prepared by treating oxyhemoglobin with potassium ferricyanid.

Fig. 5.—Methemoglobin, ox blood. Prepared by treating oxyhemoglobin with potassium ferricyanid.

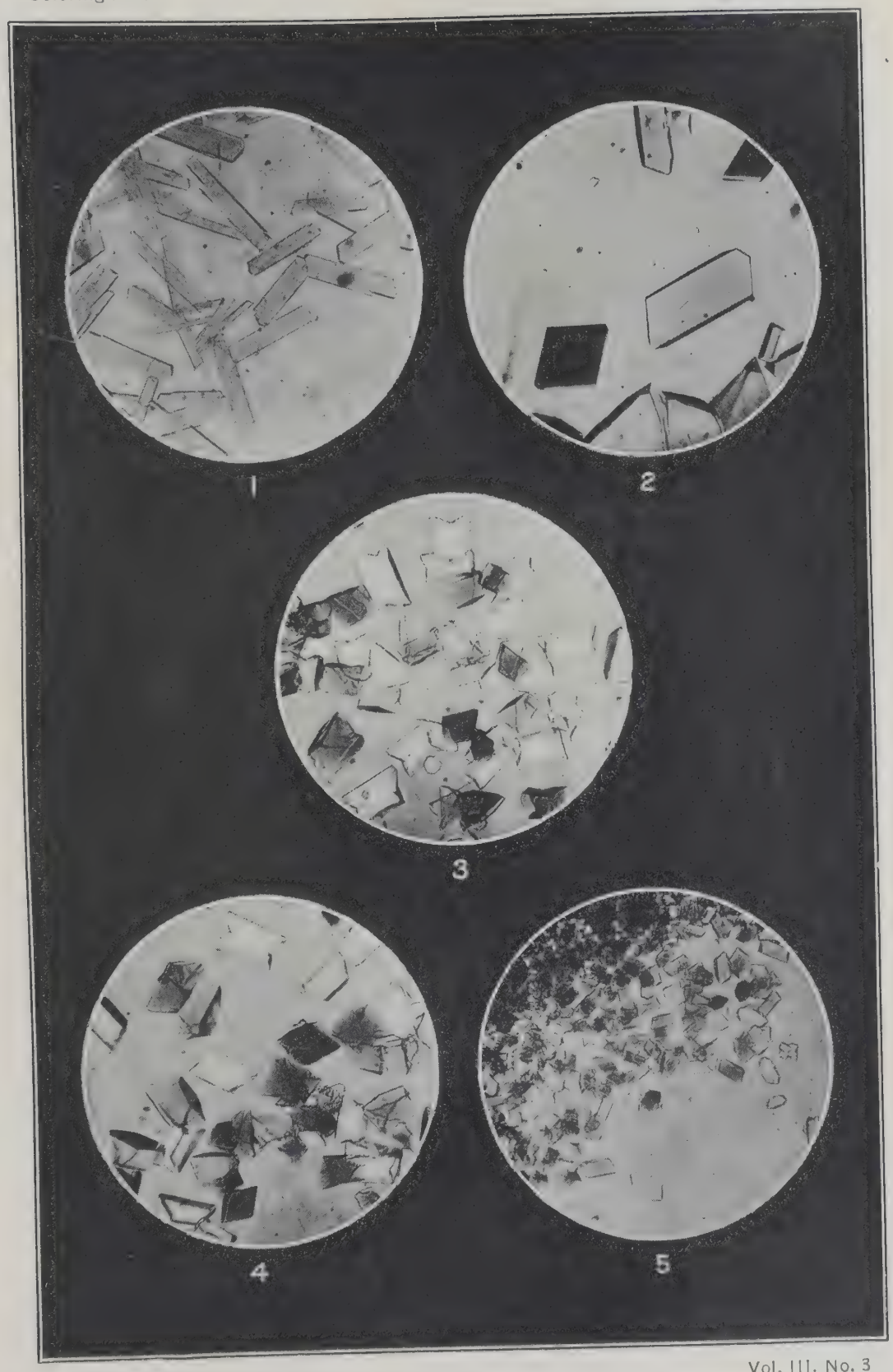
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PLATE XXXIII

Fig. 1.—Oxyhemoglobin, sheep blood.
Fig. 2.—Oxyhemoglobin, sheep blood.
Fig. 3.—NO-hemoglobin, sheep blood.
Fig. 4.—NO-hemoglobin, pig blood.
Fig. 5.—NO-hemoglobin, pig blood.

OIL CONTENT OF SEEDS AS AFFECTED BY THE NUTRITION OF THE PLANT

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INTRODUCTION

Although oils and fats are very widely distributed in the plant world, the commercial supply of these products is derived chiefly from a comparatively small number of species, and in most cases the seeds of these plants furnish the raw material. In general, the oil produced by the plant is usually stored in the seed or other reproductive parts, and for this and other reasons the seed constitutes the most favorable material for a study of the quantitative production of oil in plants.

The seed as a rule varies less in composition than other plant parts, as would be inferred when we consider its relatively small size along with the fact that it normally possesses the ability to reproduce in detail the distinctive characters of the parental type. Nevertheless, when grown under widely different conditions, seeds frequently show such marked changes in composition that their agricultural or commercial value is materially affected. The composition of certain seeds, more particularly wheat and other grains, as influenced by environment, has been extensively investigated in so far as relates to their content of protein, carbohydrate, and ash; but no extensive investigation of the oil content of seeds as affected by the various factors of nutrition has thus far been reported. There are on record, however, numerous analyses of oleaginous seeds grown in different regions, which indicate marked differences in oil content, presumably due, at least in part, to the varying conditions under which the seeds were produced. Some of our important crop plants are of value, primarily, for the oil contained in the seed; and it is a matter of practical importance to ascertain, so far as possible, the most favorable conditions for obtaining maximum yields of oil. It was with this object in view that an investigation was undertaken, some of the results of which are presented in the present paper.

The true fatty oils, composed of glycerin esters of the fatty acids, are quite different chemically from the carbohydrates; and, in fact, the two groups of compounds have little in common, except that both contain only the three elements—carbon, hydrogen, and oxygen. On the other hand, there is a very intimate and significant physiological rela-

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tionship between the carbohydrates and the oils in the seed and other parts of the plant. Outside of the living cell the transformation of carbohydrate into fat, or the reverse process, has not been accomplished; but both of these processes are readily carried out by the living protoplasm. The researches of Müntz (1886),[1] Leclerc du Sablon (1895, 1896), Gerber (1897 a, b), Ivanow (1912), and others with the poppy (*Papaver somniferum*), flax (*Linum usitatissimum*), rape (*Brassica* spp.), soy bean (*Glycine hispida*), castor-oil plant (*Ricinus communis*), walnut (*Juglans regia*), sweet almond (*Amygdalus communis*), hemp (*Cannabis sativa*), and sunflower (*Helianthus annuus*) all go to show that the development of oleaginous seeds is characterized by a progressive accumulation of oil accompanied by a corresponding decrease in carbohydrates. Under proper conditions this transformation takes place in unripe seeds detached from the mother plant, further indicating that the oil is derived from the carbohydrate. Although oleaginous seeds in general are relatively rich in protein and the accumulation of oil proceeds simultaneously with that of protein, no evidence exists that there is any direct relationship between the two processes.

From the researches of the investigators mentioned, together with those of Schulze (1910) and his pupils, it may be inferred that the plant during the period prior to blooming normally accumulates an adequate supply of nutrients, chiefly in the form of carbohydrate and protein, to insure the development of the seed. At or near the blooming stage there begins a general movement of nutrients in the form of the simpler sugars and soluble nitrogenous constituents through the stem toward the reproductive parts. During the first stages of development of the seed the carbohydrates are laid down largely in the form of cellulose and hemicellulose, in order to provide for the early development of the testa, or seed coat, which serves first as conductive tissue for the embryo and in later stages as a protective membrane but not as a depository of surplus food. Then follows in the seed of some species a marked accumulation of so-called reserve carbohydrates, mainly starch and hemicellulose, while in other species the nitrogen-free "reserve" food accumulates in the form of oil. Examination of the stem parts during the development of the seed has shown that the organic non-nitrogenous nutrients flowing into oleaginous seeds are largely the same as for starchy seeds—namely, soluble carbohydrates.

While there appears to be no doubt that the oil in the plant cell, at least in the higher plants, is derived from carbohydrate, the mechanism of the reactions involved is not understood, since the transformation is not known outside the living cell.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 249.

PROBLEMS INVOLVED AND GENERAL METHODS OF PROCEDURE

The growth, development, and composition of the individual plant depend on the combined action of heredity and environment. It follows that in any investigation of heredity or of environment in relation to plant life such methods should be followed as will make it possible to distinguish between these two forces. This principle of clearly distinguishing between the effects of environment and those due to heredity is simple enough in theory, but in practice it is often extremely difficult to follow because of interrelations between the two forces which are not fully understood. Thus, nonheritable variations in the progeny of relatively pure strains grown under what would seem to be uniform environmental conditions become especially apparent when we come to deal with quantitative differences such as are involved in a study of variable chemical composition as influenced by the factors of nutrition. In our experiments on the nicotine content of tobacco, the oil content of seeds of various species, and other quantitative characters, efforts to insure the greatest uniformity possible in environmental conditions have failed to effect anything like uniform composition in the progeny of individuals representing the purest strains available. Some of the strains of tobacco under study had been inbred for eight generations.

It follows that in dealing with quantitative differences in composition as influenced by environment a sufficiently large number of individuals must be used to avoid misleading results due to variations that can not be brought under control. Where conditions have made it impracticable to grow relatively large numbers of plants for study, the alternative of repeating the experiment has been adopted. It is equally important to use caution in reaching generalizations based on results obtained with an insufficient number of species or varieties. In the course of our work on the oil content of seeds it has been frequently observed that in the case of the soy bean, for example, different varieties are not always influenced in the same manner by the environment. In such plants as the soy bean and cotton, therefore, as many varieties as practicable have been included in the experiments. In selecting plants for study those that are of special importance by reason of the oil content of the seeds and which are otherwise adapted to the work in view have been taken. Thus far we have utilized cotton (*Gossypium* spp.), soy bean, peanut (*Arachis hypogaea*), and sunflower.

With reference to chemical composition, the commercial value of seeds evidently depends on the relative percentages of their several constituents, but from the standpoint of the grower the returns also depend on the quantity of seed produced—that is, on the number and size of the seeds produced by the individual plant or on a given acreage. Hence, in the

present investigation the weight of the seed in addition to its percentage composition was determined in order to ascertain the actual quantity of oil which it contained. When practicable, data were collected as to the size and character of growth of the plant as a whole for purposes of correlation and more particularly in dealing with specific factors of the nutrition process as a measure of the relative effects produced by the special conditions of the experiment. The actual yield of seed has not received any special attention, since this is greatly affected by factors of nutrition which have little influence on the problems involved, and, moreover, the matter of crop yields constitutes a separate problem. In analyzing the seed the official method[1] for the determination of oil was followed, using anhydrous ether as the solvent.

OIL CONTENT OF SEED AT SUCCESSIVE STAGES OF DEVELOPMENT

It has been shown that the accumulation of oil in the seed does not set in actively during the very earliest stages in the development of the seed, and the work of Ivanow (1912) suggests that there is a period of very intense oil formation, which occurs about midway between blooming and the final maturity of the seed. This raises the question as to the existence of a "critical period" in oil formation which would have an important bearing on the effects of external conditions on the quantity of oil produced. Samples of soy beans and of cotton seed were collected at different stages of maturity, to secure more definite information on this point. The weight of the seed also was obtained in each case, so as to ascertain the changes in the absolute as well as the relative oil content. To arrest respiration promptly and thereby avoid changes in composition, the immature seeds were dried in the oven at 70° to 80° C. In the case of the soy bean 5 to 6 pods were picked from each of 100 to 125 plants in taking the samples. The material was grown at the Arlington Experiment Farm, Va., the Peking soy bean in 1910 and the others in 1912. The several pickings from each variety were taken from the same plants at stated intervals, but for obvious reasons the different pickings do not necessarily represent the actual growth made by the beans in the intervals covered. They are strictly comparable, however, as to the relation between the oil content and the size of the seed at the several stages of maturity. In the case of the cotton seed care was taken to obtain the same number of immature and mature bolls from each plant, and these were always taken from the same branch, about 12 plants being used for each pair of samples. The results obtained are shown in Tables I and II.

[1] Wiley, H. W., et al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr., Bur. Chem. Bul. 107 (rev.), 272 p., 11 fig. 1908.

TABLE I.—*Oil content of soy beans gathered at various stages of maturity at Arlington Experiment Farm, Va., in 1910 and 1912*

VARIETY, S. P. I. NO. 19981

Date harvested.	Weight of 1,000 beans.	Moisture in beans.	Oil in moist beans.	Oil in 1,000 beans.	Date harvested.	Weight of 1,000 beans.	Moisture in beans.	Oil in moist beans.	Oil in 1,000 beans.
	Gm.	*Per cent.*	*Per cent.*	*Gm.*		*Gm.*	*Per cent.*	*Per cent.*	*Gm.*
Aug. 13	3.53	7.50	2.95	0.104	Sept. 11	356.8	6.60	18.05	64.4
20	39.3	5.50	10.65	4.19	16	447.0	7.55	18.02	80.5
23	96.24	5.80	13.40	12.9	21	472.3	5.95	18.60	87.8
28	146.2	6.10	15.94	23.3	25	498.7	7.44	18.37	91.6
Sept. 3	263.7	5.40	16.70	44.0	30	487.0	6.35	18.85	90.8
6	282.0	6.55	17.00	47.9	Oct. 7	479.3	7.55	17.77	85.2

VARIETY, S. P. I. NO. 21755

July 24	8.2	7.00	3.80	0.312	Aug. 13	158.0	5.80	14.75	23.3
30	32.3	5.50	9.38	3.03	20	217.4	6.00	15.83	34.4
Aug. 3	81.8	6.30	11.58	9.48	26	210.5	5.00	15.65	32.9
7	98.3	5.40	12.52	12.3					

VARIETY, S. P. I. NO. 32907

Aug. 20	3.28	6.70	3.38	0.110	Sept. 4	67.3	5.85	16.95	11.4
23	12.5	6.50	7.65	.955	7	72.1	5.70	17.20	12.4
27	23.2	5.15	12.10	2.81	11	85.0	6.00	18.10	15.4
30	49.2	5.15	14.95	7.36	20	82.5	5.55	18.28	15.1

TABLE II.—*Oil content of immature and mature cotton seed grown at Thompsons Mills, Ga., in 1910*

Variety and condition of seed.	Lint.	Weight of 1,000 seeds.	Hulls.	Meats.	Moisture in meats.	Oil in moist meats.	Oil in whole seed.	Oil in 1,000 seeds.
Toole:	*Per cent.*	*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Gm.*
Immature	41.8	78.4	44.6	55.4	3.80	37.65	20.9	16.4
Mature	38.1	93.3	38.8	61.2	4.05	38.03	23.3	21.7
Trice:								
Immature	31.8	102.0	45.6	54.4	3.72	34.60	18.8	19.2
Mature	30.8	134.4	42.4	57.6	4.00	36.17	20.9	28.2
Sam McCall:								
Immature	35.3	133.0	40.4	59.6	3.95	35.80	21.4	28.5
Mature	33.5	155.6	39.3	60.7	4.05	37.93	22.9	35.7

In all cases the relative weight of the seed rather than the date of harvesting is to be taken as the more nearly correct index of the stage of development. The first pickings of the soy beans were made when the seeds were exceedingly small, and the final pickings represent the fully matured seed. The results are definite and conclusive. Except for the period immediately following blooming and that directly preceding final

maturity, there is throughout the development of the seed a gradual and rather uniform gain in the oil content as compared with the growth of the seed. There is no evidence of a definite "critical period" for the accumulation of oil during the development of the seed. Considering only the percentage of oil, there is a very sharp increase during the first few weeks after blooming, and then only a slow gain until near the end of the ripening. During the final stage of ripening there is a decrease both in the size of the seed and in the oil content. This phenomenon, which was observed also by Müntz (1886), is probably due to continued respiratory activity after assimilation has ceased. In the case of the cotton seed the immature samples were taken when the green bolls had reached full size and had begun to show numerous brown spots. As in soy beans, the increase in oil proceeds somewhat more rapidly than the growth of the seed.

OIL CONTENT OF SEED AS AFFECTED BY RATIO OF LEAF SURFACE TO QUANTITY OF SEED PRODUCED

It has been shown that the accumulation of oil in the seed proceeds throughout the greater portion of the period of development and ripening. It also has been pointed out that during the so-called vegetative period preceding blooming there is an accumulation of carbohydrates in the leaves and stems which is later utilized in the formation of the oil deposited in the seed. From these facts it might be inferred that the total quantity of oil stored in the seed would be affected by the relative extent of the photosynthesizing plant parts, more particularly the leaves, while, on the same basis, the percentage content of oil might be influenced by the ratio between the photosynthesizing parts and the quantity of seed produced. In other words, it is to be expected that premature shedding of leaves, such as often happens under adverse conditions, or the shedding of a portion of the blossoms would affect the accumulation of oil in the seed. As regards the quantity of seed produced, a diminished supply of accumulated carbohydrate might lead to the production of a smaller number of seeds or of smaller sized seeds, or possibly both.

For the reasons indicated it is apparent that in any analytic study of oil production in the seed as related to factors of nutrition account must be taken of the possible effects of the nutrition conditions during the two principal life periods of the plant—namely, the vegetative and the reproductive. The production of oil by the plant requires favorable conditions for the accumulation of carbohydrate during the vegetative period and for the transformation of carbohydrate into oil during the second period, although there may be, of course, more or less overlapping of the two processes. As a special phase of the influence of the accumulation of carbohydrate on oil formation, experiments were carried out with soy beans in which the normal distribution of the vegetative and reproductive plant parts was modified by partial defoliation and by removal of a portion of the blossoms or very young seed pods. The number of plants used in each

experiment ranged from 35 to 50. Data were also obtained as to the effects of the two treatments on the general development of the plant, as indicated by the height, the weight of the air-dry root and stalk minus the leaves, and the total yield of seed. The results are presented in Tables III and IV.

TABLE III.—*Oil content of soy beans as affected by partial defoliation*

Variety and treatment.	Date of blooming.	Average weight of stalk and root.	Average height of plant.	Average yield of beans per plant.	Weight of 1,000 beans.	Moisture in beans.	Oil in moist beans.	Oil in 1,000 beans.
S. P. I. No. 32907:		*Gm.*	*Inches.*	*Gm.*	*Gm.*	*P. ct.*	*P. ct.*	*Gm.*
Control	July 30	33.7	22.2	56.6	89.8	7.45	16.75	15.0
Number of leaves reduced to about 40 per cent of normal on June 29, July 16, 22, 30, and Aug. 14	...do....	20.7	19.1	34.1	85.4	6.65	17.52	15.0
Number of leaves reduced to about 50 per cent of normal at same periods as above	...do....	24.0	20.4	42.2	85.6	7.40	17.80	15.2
Control	...do....	36.8	25.2	51.2	87.1	6.60	17.20	15.0
Number of leaves reduced to about 40 per cent of normal on Aug. 1 and 15	...do....	24.1	21.5	30.7	81.8	6.75	17.85	14.6
S. P. I. No. 21755:								
Control	July 8	9.7	11.1	24.5	209.0	8.65	16.21	33.9
Number of leaves reduced to about 40 per cent of normal on July 15 and 30	...do....	5.3	9.7	11.5	168.5	8.55	16.32	27.5
S. P. I. No. 30599:								
Control	...do....	24.5	27.0	61.1	179.4	6.20	19.95	35.8
Number of leaves reduced to about 40 per cent of normal on July 15 and 22	...do....	16.5	21.5	55.2	167.8	6.25	20.93	35.1
S. P. I. No. 30745:								
Control	...do....	24.5	24.1	58.6	192.6	7.95	19.80	38.1
Number of leaves reduced to about 40 per cent of normal on July 15 and 22	...do....	16.7	19.2	32.1	180.0	6.05	20.35	36.6
S. P. I. No. 30593:								
Control	...do....	27.2	24.4	59.4	190.3	6.70	20.93	39.8
Number of leaves reduced to about 30 per cent of normal on July 15 and 75 per cent Aug. 12	...do....	13.5	19.9	33.8	160.4	6.40	21.30	34.2

Considering, first, the effects of partial defoliation (Table III), in all cases the weight of the root and stalk, the height of the plant, and the total yield of beans are decidedly reduced. The size of the beans, however, is only slightly reduced, so that the decreased yield is due almost entirely to the smaller number of beans developed. It is an interesting fact that the small decrease in size of the beans is almost exactly offset by the increase in percentage of oil, so that the actual quantity of oil in the individual seed remains practically the same. This fact only holds apparently within certain limits, for in the case of the variety designated as "S. P. I. No. 30593," where the defoliation was nearly three-fourths complete, the decrease in size of the bean was too great to be fully offset by the higher percentage of oil. The most striking exception, however, is shown by S. P. I. No. 21755. This variety differs from the others in that the seed are matured a very short time after blooming. In the present case the seed were fully ripe on August 26, whereas

all of the remaining varieties were about five weeks later in reaching full maturity. It appears, therefore, that a degree of defoliation which but slightly modified the size and oil content of the seed in those varieties requiring a long period for the development of the seed brought about a much more decided effect in the variety which is able to fully develop rather large-sized seed in a very short period of time. Another reason for the greater effect of the defoliation on the size of the bean, as well as on the total yield of beans, in S. P. I. No. 21755 is that the foliage normally is less abundant than that of the other varieties.

TABLE IV.—*Oil content of soy beans as affected by partial removal of very young seed pods*

Variety and treatment.	Date of blooming.	Average weight of stalk and root.	Average height of plant.	Average yield of beans per plant.	Weight of 1,000 beans.	Moisture in beans.	Oil in moist beans.	Oil in 1,000 beans.
		Gm.	*Inches.*	*Gm.*	*Gm.*	*P. ct.*	*P. ct.*	*Gm.*
S. P. I. No. 32907:								
Control	July 30..	36.3	25.7	50.2	84.1	6.45	17.15	14.4
Large number of pods removed on Aug. 19	...do....	49.7	27.2	55.0	103.1	6.65	17.69	18.2
S. P. I. No. 21755:								
Control	July 8...	9.7	11.1	24.5	209.0	8.65	16.21	33.9
Larger portion of pods removed on July 15 and 22	...do....	9.0	10.8	9.8	204.6	8.00	15.83	32.4
S. P. I. No. 30599:								
Control	...do....	24.5	27.0	61.1	179.4	6.20	19.95	35.8
Larger portion of pods removed on July 15 and 22	...do....	36.2	21.5	52.6	202.2	6.40	20.02	40.4
S. P. I. No. 30745:								
Control	...do....	24.5	24.1	58.6	192.6	7.95	19.80	38.1
Larger portion of pods removed on July 15 and 20 and Aug. 19	...do....	38.7	24.2	39.1	227.8	6.35	19.15	43.7
S. P. I. No. 30593:								
Control	...do....	27.2	24.4	59.4	190.3	6.70	20.93	39.8
More than two-thirds of pods removed on July 15 and 20 and Aug. 12	...do....	42.1	23.3	25.7	244.4	6.50	19.95	48.8

It might be expected that the effects produced by removing a portion of the young seed pods would be largely the reverse of those produced by partial defoliation, and this is found to be true in part. Removing a portion of the pods resulted in much heavier root and stalk. The effect on yield of the reduction in the number of beans allowed to develop is offset to a considerable extent by a notable increase in the size of the beans. The increase in the size of the bean is not associated with a corresponding decrease in the percentage of oil; hence, the actual quantity of oil in the individual seed is considerably increased. Here, again, the early-maturing variety, S. P. I. No. 21755, stands out as an exception. Reducing the number of seed allowed to develop failed to increase the weight of the vegetative parts or the size of the seed and its oil content.

Considering the two sets of experiments together, when the development of the seed extends through a relatively long period, a reduction of, say, 50 per cent in the normal proportion of leaves or photosynthetic organs leads to a decreased weight of the other vegetative parts, as well as of the total yield of seed, but the size of the seed is only slightly re-

duced, and the quantity of oil in the individual seed is scarcely changed. A reduction in the normal proportion of the reproductive parts, on the other hand, leads to an increase in weight of the vegetative parts, and the size of the seed and its oil content are materially increased. These facts are not applicable to the variety which developed its seed within a short period of time.

OIL CONTENT AS AFFECTED BY SIZE OF SEED

In studying quantitative relationships of seeds one is at once confronted with the fact that in any particular lot of seed there is always considerable variation in the size of individuals, whatever the conditions under which the seed may be grown. This is true even of the seed from an individual plant. To ascertain whether there is any constant relationship between the oil content and the size of the seeds from the same plant, seeds of several varieties of soy beans grown in different localities were separated by hand into the larger and smaller sizes and their oil content was determined. The results are shown in Table V.

TABLE V.—*Oil content of soy beans of large and small size from the same plant*

Variety and locality.	Size of beans.	Weight of 1,000 beans.	Moisture in beans.	Oil in moist beans.
		Gm.	*Per cent.*	*Per cent.*
Ogemaw (S. P. I. No. 17258):				
Pullman, Wash.	Large.	171	5.60	13.88
Do.	Small.	106	5.25	14.17
Amherst, Mass.	Large.	290	6.30	16.92
Do.	Small.	169	6.60	16.63
Wooster, Ohio.	Large.	259	6.40	16.30
Do.	Small.	137	6.40	15.95
Hansen (S. P. I. No. 20409):				
Pullman, Wash.	Large.	46	6.10	11.72
Do.	Small.	25	5.85	12.27
Statesville, N. C.	Large.	64	7.35	13.27
Do.	Small.	37	6.80	13.50
La Fayette, Ind.	Large.	86	6.75	11.72
Do.	Small.	53	6.75	11.15
Buckshot (S. P. I. No. 17251):				
Kingston, R. I.	Large.	364	6.30	17.45
Do.	Small.	196	6.65	17.00
Amherst, Mass.	Large.	389	6.05	17.87
Do.	Small.	215	6.40	16.95

Considering only the large and small beans from the same lot of seed, it appears that generally the percentage of oil is approximately the same, although there are some cases in which there are considerable differences. No fixed rule can be laid down as to the relative percentages of oil in large and small beans. It is evident that there are marked differences in the size of the beans in each lot, but a quantitative separation is impracticable, since there are all gradations in size. The only practicable method of comparing different lots of seed, therefore, is to secure average values based on comparatively large quantities, simply counting out the seed as they come, without any attempt at separation into sizes.

OIL CONTENT AS AFFECTED BY LENGTH OF GROWING PERIOD

Independently of any differences between early and late varieties as such, it might be expected that difference in oil content would be influenced by both the character and the length of the growing period. Several investigators have concluded that the length of the growing period is an important factor in determining the starch and protein content of wheat. In plants of determinate growth, in which all the seed are developed during approximately the same period, the effect of the length of the growing period on the oil content may be studied by making successive plantings at given intervals, since many of such species show a marked tendency to shorten the growing period when planted abnormally late. Mooers (1909) has called attention to this tendency in soy beans, and the present authors have determined the oil content in the seed of several varieties of this crop planted at stated intervals during the spring and summer months.[1] The results of the tests are shown in part in Table VI.

TABLE VI.—*Oil content of soy beans planted at intervals of two weeks in 1911*

Variety and date of planting.	Number of days from planting to blooming.	Number of days from blooming to full maturity.	Number of days from planting to full maturity.	Weight of 1,000 beans.	Moisture in beans.	Oil in beans.	Oil in 1,000 beans.
S. P. I. No. 21755:				*Gm.*	*Per cent.*	*Per cent.*	*Gm.*
May 1	40	62	102	186	6.90	17.25	32.4
May 15	35	58	93	153	7.05	16.38	25.1
June 1	37	50	87	166	6.90	17.40	28.9
June 15	32	54	86	145	7.10	17.27	25.0
July 1	27	58	85	168	7.60	15.51	26.1
Haberlandt (S. P. I. No. 17271):							
May 1	61	83	144	210	6.65	19.73	41.4
May 15	54	76	130	211	6.70	19.47	41.1
June 1	50	74	124	217	6.35	20.32	44.1
June 15	45	74	119	240	6.80	18.95	45.5
July 1	44	72	116	244	7.20	17.35	42.3
Buckshot (S. P. I. No. 17251):							
May 1	40	72	112	271	6.40	19.15	51.9
May 15	40	69	109	272	6.40	19.62	53.4
June 1	39	68	107	277	6.20	19.35	53.6
June 15	39	58	97	250	6.10	18.95	47.4
July 1	33	64	97	294	6.40	18.43	54.2
Medium Yellow (S. P. I. No. 17269):							
May 1	56	102	158	329	6.45	17.60	57.9
May 15	49	94	143	313	6.25	18.25	57.1
June 1	50	77	127	312	5.70	17.75	55.4
June 15	45	73	118	305	5.80	17.38	53.0
July 1	41	66	107	334	6.30	16.43	54.9

[1] In these experiments the authors are indebted to the Office of Forage-Crop Investigations, of the Bureau of Plant Industry, for samples of the soy beans which were grown at the Arlington Experiment Farm, Va., together with the data as to time of planting and date of maturity.

The different plantings of soy beans show marked variations in the size of the beans and in their oil content, but there is no definite relationship between these characters and the date of planting. In other words, the character rather than the length of the season in which the seed is developed seems to be the important factor. The seed from the latest plantings contain a somewhat lower percentage of oil than the others, but this relationship is not verified in other tests which have been omitted from Table VI for the sake of brevity. These additional data all show lack of definite relationship between size and oil content of the seed and the length of the growing period. There is a remarkable difference between varieties as to the shortening of the period required for maturing seed when planted late, as illustrated by the Buckshot and the Medium Yellow varieties.

VARIETAL DIFFERENCES IN OIL CONTENT OF THE SEED

As a preliminary to the study of oil content as affected by nutrition, it was necessary to ascertain the relation of heredity to the quantity of oil produced in the seed in so far as relates to the comparative behavior of different varieties. This work has been limited largely to soy beans and cotton, since collections of varieties of the other oil-producing plants investigated have not been available.

The method of procedure has been to grow a number of varieties of each species under uniform conditions, to use the purest seed obtainable, and to repeat the tests for several seasons, using the precautions which have been discussed above in drawing samples for analysis. In Table VII are given the results obtained from material furnished by the Office of Forage-Crop Investigations, representing seven varieties of soy beans grown at the Arlington Experiment Farm in 1907, 1908, and 1910.

TABLE VII.—*Varietal differences in the oil content of soy beans grown at Arlington Experiment Farm, Va., in 1907, 1908, and 1910.*

Variety and year grown.	Weight of 1,000 beans.	Moisture in beans.	Oil in moist beans.	Oil in 1,000 beans.
Shanghai (S. P. I. No. 14952):	*Gm.*	*Per cent.*	*Per cent.*	*Gm.*
1907	215.4	6.65	19.55	42.1
1908	186.1	6.16	18.37	34.2
1910	217.1	6.80	20.20	43.8
Average	206.2	6.54	19.37	40.0
Eda (S. P. I. No. 17257):				
1907	280.6	6.30	19.70	53.5
1908	263.4	6.17	19.90	52.3
1910	269.4	6.15	21.55	58.2
Average	271.1	6.21	20.38	54.7

TABLE VII.—*Varietal differences in the oil content of soy beans grown at Arlington Experiment Farm, Va., in 1907, 1908, and 1910*—Continued

Variety and year grown.	Weight of 1,000 beans.	Moisture in beans.	Oil in moist beans.	Oil in 1,000 beans.
	Gm.	*Per cent.*	*Per cent.*	*Gm.*
Yosho (S. P. I. No. 17262):				
1907	182. 2	6. 90	16. 78	30. 6
1908	170. 2	6. 35	16. 25	27. 7
1910	202. 8	6. 65	15. 44	31. 3
Average	185. 0	6. 63	16. 16	29. 9
Amherst (S. P. I. No. 17275):				
1907	193. 6	6. 65	20. 15	39. 1
1908	179. 3	5. 50	19. 50	34. 9
1910	176. 0	6. 05	20. 33	35. 7
Average	182. 9	6. 07	20. 00	36. 6
S. P. I. No. 19981:				
1907	335. 8	6. 60	20. 67	69. 6
1908	311. 4	4. 85	20. 20	62. 8
1910	331. 9	6. 45	21. 86	72. 8
Average	326. 0	5. 97	20. 91	68. 4
S. P. I. No. 20410:				
1907	56. 9	6. 80	15. 67	8. 9
1908	63. 4	6. 18	15. 55	9. 9
1910	64. 6	6. 40	15. 45	10. 0
Average	61. 6	6. 46	15. 56	9. 6
S. P. I. No. 22312:				
1907	224. 2	7. 30	17. 72	39. 5
1908	185. 3	4. 93	16. 00	29. 6
1910	214. 1	6. 65	18. 90	40. 4
Average	207. 9	6. 29	17. 54	36. 5

In Table VIII are shown the results from six varieties of Upland cotton, also a foreign species (Hawasaki) grown in the Piedmont section of Georgia and the Coastal Plain region of South Carolina. The data presented are the averages for the years 1909, 1910, and 1911 in these two sections, and in each case all plantings were from the same original lot of seed.

TABLE VIII.—*Varietal differences in the oil content of cotton seed grown in northern Georgia and in the Coastal Plain region of South Carolina*

[Average for three years]

Variety of cotton.	Cotton seed grown in—											
	Northern Georgia.						Coastal Plain region of South Carolina.					
	Lint.	Weight of 1,000 seeds.	Hulls.	Moisture in kernels.	Oil in moist kernels.	Oil in 1,000 seeds.	Lint.	Weight of 1,000 seeds.	Hulls.	Moisture in kernels.	Oil in moist kernels.	Oil in 1,000 seeds.
	Per cent.	*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*	*Gm.*
King	36.8	103.8	40.5	4.98	36.54	22.6	36.7	96.9	41.8	4.79	39.14	21.7
Russell	31.5	154.5	42.4	5.13	37.40	32.9	33.2	139.2	44.3	4.89	40.73	31.3
Shine	32.7	108.2	41.3	4.93	37.52	23.4	34.1	95.7	44.1	4.54	41.23	21.8
Toole	36.8	93.1	39.7	4.98	37.33	20.7	38.5	85.2	42.1	4.66	40.87	20.1
Dixie	32.9	111.0	40.6	4.71	38.06	25.2	33.7	100.4	42.7	4.69	42.07	24.8
Hawkins	35.0	110.8	42.2	4.76	36.72	23.7	34.2	99.0	46.4	4.56	40.83	21.0
Average	34.3	113.6	41.1	4.92	37.26	24.8	34.9	102.8	43.6	4.69	40.81	23.5
Hawasaki	41.1	73.1	51.4	6.01	30.73	10.9	41.0	63.0	52.1	4.93	32.25	9.8

The data in Table VII show that there are enormous varietal differences in soy beans both as to size of seed and as to oil content. Furthermore, it should be noted that the seasonal effects of the three years did not influence the several varieties alike with respect to either of these two characters. More extensive tests through a period of five years and with several additional varieties fully confirm these results. It is clear, therefore, that in soy beans heredity is a very important factor, not only with respect to the size and the oil content of the seed but also as regards the extent to which these characters respond to change in environment. The results with cotton, as shown in Table VIII, are quite different. There are marked varietal differences in size of seed and other important characters, but the percentage of oil is remarkably constant when the environmental conditions are the same. Williams (1906) obtained somewhat greater variations in oil content in a test with 21 varieties.

EXTENT TO WHICH THE ENVIRONMENT MAY AFFECT THE OIL CONTENT

Before entering upon a study of the individual factors of nutrition in their relation to the formation of oil in the plant, it was desired to obtain some idea as to the extent to which the quantity of oil accumulating in the seed may be influenced by change in the general environment. Some varieties of soy beans may be grown under a very wide range of conditions, and for this reason this plant was largely used in the experiments. Through the cooperation of the Office of Forage-Crop Investigations several varieties of soy beans were grown by a number of the State experiment stations, and samples of the seed were subjected to analysis. The results of the experiments are given in Table IX.

TABLE IX.—*Oil content of soy beans grown under different environmental conditions*

Variety and locality.	Weight of 1,000 beans.	Moisture in kernels.	Oil in kernels.	Oil in 1,000 beans.
	Gm.	*Per cent.*	*Per cent.*	*Gm.*
Hansen (S. P. I. No. 20409):				
Wooster, Ohio	50.1	5.15	11.90	6.0
Statesville, N. C.	51.0	5.10	12.95	6.6
Pullman, Wash.	39.4	5.95	12.25	4.8
La Fayette, Ind.	72.7	5.15	11.05	8.0
Auburn, Ala.	50.9	6.40	12.38	6.3
Kingston, R. I.	64.5	6.05	12.00	7.7
Buckshot (S. P. I. No. 17251):				
Wooster, Ohio	251.2	5.85	16.40	41.2
Pullman, Wash.	156.3	5.80	14.55	22.7
La Fayette, Ind.	334.6	6.10	13.25	44.3
Auburn, Ala.	250.4	5.75	20.60	51.6
Kingston, R. I.	347.5	5.70	18.00	62.6
Guelph (S. P. I. No. 17261):				
Wooster, Ohio	164.0	5.82	16.20	26.6
Statesville, N. C.	269.0	6.12	20.05	53.9
La Fayette, Ind.	190.9	5.32	18.40	35.1
Auburn, Ala.	182.4	5.90	20.90	38.1
Kingston, R. I.	196.1	5.00	17.65	34.6
Ogemaw (S. P. I. No. 17258):				
Wooster, Ohio	207.4	5.70	16.45	34.1
Statesville, N. C.	193.5	5.15	17.05	33.0
Pullman, Wash.	137.8	5.50	14.40	19.8
La Fayette, Ind.	249.6	6.00	13.70	34.3
Auburn, Ala.	233.3	5.80	19.25	44.9
Kingston, R. I.	235.6	6.05	17.00	40.0

In some cases there are differences of more than 100 per cent in the size of the seed, and also very large differences in the percentage of oil, when soy beans are grown in different localities. It is evident that environment, as well as heredity, may affect tremendously the size of the seed and the quantity of oil stored therein. It should be noted again, however, that the behavior of the four varieties of soy beans was by no means the same when grown in different localities. There seems to be one exception to this observation—namely, that the conditions at Pullman, Wash., were such as to produce in each case abnormally small seed.

As cotton does not thrive in a cool climate, it has not been practicable to study the development of oil in the seed under such a wide range of conditions as in the case of soy beans. It can not be stated, therefore, whether the quantity of oil stored in the seed is subject to as wide fluctuations as have been noted in soy beans. By reference to Table VIII it will be seen that in a 3-year test all of the six varieties of cotton produced considerably heavier seed, containing a decidedly lower percentage of oil, when grown in the Piedmont section of northern Georgia than when grown in the Coastal Plain region of South Carolina. The increase in size of seed in northern Georgia was not entirely offset by the decrease in percentage of oil, so that the

actual quantity of oil stored in the seed produced under these conditions was somewhat greater than in those grown in the Coastal Plain region. There was also considerable yearly fluctuation in the oil content of the seed in both sections, owing to varying seasonal conditions. (See Table X.) As already noted, the uniformity in behavior of all the varieties of cotton contrasts sharply with the varietal differences observed in soy beans. The average difference in oil content for the six varieties of cotton as grown in the two localities is greater than the varietal differences in either locality.

OIL CONTENT OF SEED AS AFFECTED BY SOIL

In dealing with the response of the plant to differences in the environment, it is frequently sought to differentiate between the effects of climate and those ascribed to the soil. Climate, or the average weather, as ordinarily understood, refers to conditions of the atmosphere, of which temperature and moisture are perhaps the most important as factors of nutrition. But the soil is likewise subject to variation in temperature and moisture, and there must be a tendency toward equilibrium in temperature and moisture between these two media in which the plant lives.

It is true that under any fixed weather, or climatic, conditions plants grown on contrasted soil types may show well-defined differences in their development, but such relationships are subject to change with any change in the climatic factors. There is ample evidence to show that the differences in plant development observed on contrasted soil types during one season may be completely reversed in another season. Again, it is true that in extreme cases differences in climate may produce certain definite differences in plant development more or less independently of the soil type. Within ordinary ranges of soil and climatic differences, however, it is hardly possible to develop far-reaching generalizations as to the specific effects of either independently of the other, for change of climate results in a change of soil conditions, and vice versa.

In spite of the above-mentioned limitations, which must apply in considering soil and climate as environmental factors, it seemed desirable to obtain data as to the influence of differences in soil type on the accumulation of oil in the seed as produced under varying seasonal conditions. In the experiments with cotton six representative Upland varieties were grown for three consecutive years (1909–1911) at Thompsons Mills, Ga., on two adjoining but contrasted types of soil, the original lots of seed being used for each year's planting. For convenience these soil types are designated as "red soil" and "gray soil," respectively. Both belong to the Cecil series and were in a good state of cultivation. The red soil is a comparatively heavy, tenacious clay, while the gray soil is an open-textured sandy loam. As all of the varieties were affected in a

similar manner, the results of the experiments as given in Table X are averages for the six varieties.

TABLE X.—*Average oil content of six varieties of cotton seed grown for three seasons on two different soil types at Thompsons Mills, Ga.*

Year in which grown.	Lint.		Weight of 1,000 seeds.		Hulls of seed.		Moisture in kernels.		Oil in moist kernels.		Oil in 1,000 seeds	
	Red soil.	Gray soil.	Red soil.	Gray soil.	Red soil.	Gray soil.	Red soil.	Gray soil.	Red soil.	Gray soil.	Red soil.	Gray soil.
	Per cent.	*Per cent.*	*Gm.*	*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Gm.*	*Gm.*
1909	34.2	35.3	114.2	109.7	41.3	41.7	4.15	4.12	37.09	36.40	24.2	22.3
1910	32.0	33.0	121.5	115.9	40.3	41.2	4.08	4.02	36.07	38.53	20.8	20.4
1911	35.2	36.0	110.0	107.4	40.8	41.4	0.50	0.44	37.41	37.33	24.4	23.3
Average..	34.0	35.0	115.2	111.0	40.8	41.4	4.91	4.80	37.28	37.44	25.1	24.0

Taking the average results for the three years, the red soil gave only slightly heavier seed, with a somewhat smaller proportion of hulls than the gray soil, and there was practically no difference in the oil content. Each year the seed was heavier and contained a smaller proportion of hulls on the red soil than on the light soil, but the case is quite different as regards the oil content. In 1909 the oil content was considerably higher on the red soil than on the gray, while in 1910 these relations were reversed and in 1911 the differences practically disappeared. In other words, the comparative effects of the two soil types depend on the seasonal conditions, and it so happened that there was a balancing effect for the three years covered by the experiment.

Extensive data have been accumulated as to the effect of soil type on the oil content of soy beans, but these data are too voluminous to present in detail, and only a summary of the results can be given here. In 1911 plantings of six varieties of soy beans were made on the two above-mentioned soil types at Thompsons Mills, Ga., and data were secured as to the relative oil content of the beans. The data given in Table XI are the averages of three plantings, made on May 6, June 3, and June 21, respectively, for the six varieties designated as S. P. I. Nos. 17254, 17263, 17857, 18227, 19984, and 20892. In 1912 five varieties were grown on a heavy clay soil at the Arlington Experiment Farm and on an infertile sandy soil containing a large percentage of coarse sand. The latter soil is composed largely of material dredged from the Potomac River and is designated as Potomac Flats soil. These two soils are only about half a mile apart, so that the weather conditions during the growing season were essentially the same. While, as stated, five varieties were used in this experiment, data for only two of these, S. P. I. Nos. 30599 and 30745, as a basis for comparison with the greenhouse experiment next described, are given in Table XI.

In addition to these field experiments with the soy bean, a number of tests on different soil types were made under specially controlled conditions. In the spring of 1912 the two varieties designated as S. P. I. Nos. 30599 and 30745 were grown on the above-mentioned heavy clay and Potomac Flats soils placed in the greenhouse at the Arlington Experiment Farm. The two soils occupied adjoining portions of the same bench, received the same quantities of water, and were exposed to the same conditions of light, etc. In 1911 and 1912 soy beans were grown in different soil types contained in large earthen pots set into the soil. These pots consisted of glazed tiles 3 feet long and 18 inches in diameter. A perforated bottom of concrete was set in each tile cylinder 3 inches from the lower end. The tiles were then set into the soil so that the upper ends extended 3 inches above the ground level. The cylinders were filled to the ground level with the different soils. Series of tiles were thus installed in the clay soil of the Arlington Farm and in the Norfolk fine sandy loam in the vicinity of Manning, S. C. At each location one-half the total number of cylinders were filled with the native soil and the second half with soil transported from the other point. In preparing the soils for the tiles the surface soil and the subsoil were collected separately and each lot very thoroughly mixed. In each case a sufficient quantity of the native soil thus prepared was shipped in bags to the other point, so that presumably at each point there was a series of insulated cores of two very different soil types embedded in the native soil. Apparently the insulation from the surrounding soil should be practically perfect, except possibly as regards temperature, and a special experiment, in which a cylinder was inclosed in a second one of much larger size and filled with the same soil, indicated that the temperature of the surrounding soil had but little effect on that contained in the cylinder. The two soil types, of course, would be exposed to exactly the same weather conditions in any particular locality. A second series of cylinders was installed at the Arlington Farm and filled with the native clay soil and with Hartford fine sandy loam from South Windsor, Conn. In 1912 an additional set of cylinders, filled with the Potomac Flats soil, was used in connection with the first series.

In the first series of cylinders the variety designated as S. P. I. No. 17852B was used in 1911, while S. P. I. No. 32907 was used in 1912. In the second series S. P. I. No. 21755 was used. The results of the various tests with soy beans are summarized in Table XI. Each test is complete in itself and of course can not be compared directly with the others, since, with one exception, different varieties were used and the climatic conditions were not the same. The results of each separate test are based on data from a considerable number of individuals of each variety, usually 50 to 100, and in most cases are the averages from several varie-

ties. In all cases where two or more varieties are averaged the direction of the soil effect was the same for each of the varieties, though the extent of this effect was not the same.

TABLE XI.—*Oil content and related data of soy beans and peanuts grown on different soil types*

Locality, conditions under which grown, and kind of soil.	Soy beans.						Peanuts.			
	Dry weight of 100 stalks and roots.	Yield of beans from 100 plants.	Weight of 1,000 beans.	Moisture in beans.	Oil in moist beans.	Oil in 1,000 beans.	Weight of 1,000 seed.	Moisture in seed.	Oil in moist seed.	Oil in 1,000 seed.
Thompsons Mills, Ga.:										
Field experiment—	*Gm.*	*Gm.*	*Gm.*	*P. ct.*	*P. ct.*	*Gm.*	*Gm.*	*P. ct.*	*P. ct.*	*Gm.*
Gray land	.....	.....	144.4	4.41	20.44	29.5	553.1	3.07	47.25	261.6
Red land	.....	.....	136.3	4.20	21.68	29.5	540.7	2.80	50.70	274.3
Arlington, Va.:										
Field experiment—										
Arlington clay	2,450.0	5,911.0	186.0	7.20	19.85	36.9	356.0	4.40	47.85	170.5
Potomac Flats	301.0	636.0	130.9	8.02	21.00	27.5	411.0	3.55	47.54	168.6
Greenhouse experiment—										
Arlington clay	325.0	856.0	184.1	6.02	19.88	36.6	.....	.....	.....	.....
Potomac Flats sand	351.0	561.0	169.3	6.10	19.52	33.0	.....	.....	.....	.....
Pot experiments, Series I, 1911—										
Arlington clay	.....	34.5	55.0	4.90	17.44	9.6	470.0	3.12	51.58	242.5
Norfolk fine sandy loam	.....	70.8	65.3	5.10	16.62	10.8	511.0	3.35	49.16	251.4
Pot experiments, Series I, 1912—										
Arlington clay	908.0	687.0	68.1	7.02	18.55	12.7	478.0	3.90	50.25	240.4
Norfolk fine sandy loam	610.0	562.0	89.6	7.62	15.32	13.7	509.0	3.45	46.07	234.6
Potomac Flats sand	682.0	416.0	88.7	7.36	14.89	13.2	473.0	4.15	46.12	218.1
Pot experiments, Series II, 1912—										
Arlington clay	188.0	230.0	132.6	7.62	16.57	22.0	.....	.....	.....	.....
Hartford fine sandy loam	326.0	485.6	145.1	7.36	15.22	22.1	.....	.....	.....	.....
Manning, S. C.:										
Pot experiments, Series I, 1911—										
Arlington clay	.....	57.6	95.1	5.10	16.76	16.0	465.5	3.43	49.97	233.0
Norfolk fine sandy loam	.....	64.8	87.7	5.05	16.50	14.5	480.5	3.47	49.80	239.5
Pot experiments, Series I, 1912—										
Arlington clay	.....	.....	94.1	6.45	16.69	15.7	377.4	3.66	47.62	179.5
Norfolk fine sandy loam	.....	.....	96.3	6.50	16.83	16.2	365.8	3.73	47.04	172.0

These tests include a wide range of soil types and climatic conditions, and the results as a whole emphasize the fact that the relative effects of different soil types are not specific and constant but depend largely on seasonal conditions, as was brought out in the experiments with cotton. The results in the field experiment at Arlington Farm as compared with those obtained in the greenhouse with the same soils illustrate this point. In the field test the plants suffered considerably from drought during the growing season, and here the sandy soil gave decidedly smaller beans and higher relative oil content than the clay soil. In the greenhouse the difference in size of beans largely disappeared, while the clay loam gave a somewhat higher percentage of oil than the sandy soil. In the pot experiments at Arlington Farm the lighter soils gave somewhat larger seed with lower percentages of oil than the heavier clay soil, but at Manning, S. C., there were no significant differences.

Experiments similar to those with soy beans were made with the peanut of the variety known as Spanish, and the results are given in Table XI. The effects produced by the different soil types are of the same general character as with soy beans, although the behavior of the two species under similar conditions is not always the same. In 1913 a series of pot cultures with the sunflower were carried out at the Arlington Farm in the same manner as described for soy beans, using a number of different soils in the test. The weights in grams per 1,000 seeds as grown in the Arlington clay, Norfolk sandy loam, Potomac Flats soil, Norfolk sand, and the so-called Benning sand were 90.5, 73, 79.5, 56.4, and 52, respectively, while the corresponding percentages of oil in the kernels were 51.25, 50.30, 55.70, 51.26, and 49.10. In this case soil differences brought about very marked differences in size of the seed, but the variations in relative oil content were less decided.

OIL CONTENT OF SEED AS AFFECTED BY CLIMATE

On comparing the data given in Table IX with those in Table XI it becomes apparent that the variations in the size of seed and the oil content of soy beans attributable to differences in soil type are far less than those observed when both soil and climate differ. The same relationships are observed in cotton seed, as shown in Tables VIII and X. These results are interpreted as indicating that under practical conditions climate is a more potent factor than the soil in modifying the size of seed and its oil content. The most probable explanation is that the atmosphere is subject to greater and more rapid variations in moisture and particularly in temperature, and also that the "soil climate" is greatly influenced by the weather conditions. Temperature and moisture differences of both soil and atmosphere are among the important factors of environment which may influence the plant characters under study, and this factor-complex must be at least partially analyzed before satisfactory conclusions can be reached as to the principal external factors concerned in oil formation in the plant.

OIL CONTENT OF SEED AS AFFECTED BY FERTILIZERS

The experiments with different soil types previously described have included soils varying greatly in fertility, as indicated by the comparative growth of the plants shown in Table XII, and the results as a whole show that within the limits ordinarily met with in farm practice the relative fertility of the soil does not very greatly influence the size of the seed or its oil content. A large number of fertilizer tests with cotton were carried out at Lamar and Timmonsville, S. C., in 1909, 1910, and 1911, to obtain more accurate information as to the effects of fertilizers on the size and oil content of the seed. The data are too voluminous to present in detail, but in Table XII a summary of the results for 1911

is given. In each series the results are averages of duplicate plots, except for the controls, which represent the averages for four plots in each case, all plots being one-fortieth of an acre in area. The tests in 1909 and 1910 included plots receiving four different quantities of nitrogen, four of phosphoric acid, and three of potash. Dried blood, acid phosphate, and muriate of potash were used as fertilizers.

TABLE XII.—*Results of tests with cotton at Manning, S. C., to determine the influence of fertilizers on the oil content of the seed*

Plot series No.	Plant food elements applied per acre.			Yield of seed cotton per acre.	Lint.	Weight of 1,000 seeds.	Hulls of seed.	Oil in kernels.
	Nitrogen.	Phosphoric acid.	Potash.					
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Per cent.*	*Gm.*	*Per cent.*	*Per cent.*
1	0	0	0	530	36.2	118	47.1	33.56
2	30	90	20	1,070	37.6	130	44.9	37.48
3	60	90	20	880	34.2	130	45.0	33.85
4	0	0	0	525	36.9	120	46.8	32.99
5	30	90	40	1,265	35.9	135	44.3	38.07
6	60	90	40	1,410	34.4	139	43.9	36.40
7	0	0	0	483	36.9	121	47.3	34.38
8	30	90	60	1,160	35.9	134	45.8	38.86
9	60	90	60	1,320	34.6	137	44.0	36.78
10	0	0	0	502	36.1	122	47.6	33.49
11	30	150	60	1,030	35.8	136	43.2	38.60
12	30	150	40	1,090	36.1	136	44.7	37.48
13	60	150	40	1,220	35.2	138	43.6	36.65

The soil used in 1911 was very poor, as shown by the large increases in crop yields produced by the complete fertilizers. The addition of all three elements, nitrogen, phosphorus, and potassium, combined in varying proportions, gave in all cases considerably heavier seeds, with a smaller percentage of hulls and a higher oil content in the kernels as compared with the controls. With respect to the varying quantities of the three fertilizer elements, increased applications of nitrogen had no appreciable effect on the weight of the seed and only a slight effect on the percentage of hulls, but lowered considerably the oil content of the kernels. Increased applications of phosphorus and potassium did not materially affect any of these characters. The tests of the two preceding years gave similar results.

Pot-culture tests were made in 1911 with the Peking variety of soy beans, using the tile cylinders, previously described, filled with the Arlington clay soil. In a series in which phosphorus and potassium in a fixed ratio were added in three different quantities, the yields of beans were greatly increased and the weight of the seed was not changed, while the oil content was increased about 20 per cent. With

the addition of phosphorus alone, very much the same results were obtained; but with the addition of potassium alone there was only a small increase in yield and practically no increase in oil content. In similar tests with Spanish peanuts phosphorus gave a large increase in yield and slightly increased the weight of the peas, but had no effect on the oil content. Potassium had practically no effect on the yield, the weight of seed, or the oil content.

SUMMARY

Experiments with soy beans have shown that, except for the period immediately following blooming and that directly preceding final maturity, there is a fairly uniform increase in oil content, both relative and absolute, throughout the development of the seed, and no evidence was found that there is a critical period of very intense oil formation at any stage of seed development. Tests with cotton likewise indicate that the increase in oil proceeds somewhat more rapidly than the increase in the weight of the seed.

As a consequence of the physiological relationship of oil to carbohydrate, it appears that maximum oil production in the plant requires conditions of nutrition favorable to the accumulation of carbohydrate during the vegetative period and to the transformation of carbohydrate into oil during the reproductive period. As a special phase of this relationship between carbohydrate supply and oil formation in soy beans, it was found that when the normal distribution of the vegetative and reproductive plant parts was modified by partial defoliation (50 to 60 per cent) the yield of beans was decidedly reduced, but the size of the beans and their oil content were only slightly affected, except in the case of an early-maturing variety. On the other hand, the removal of a portion of the blossoms or young pods caused a notable increase in the size of the beans allowed to develop, but did not materially affect the percentage oil content.

There is always lack of uniformity in the size of the seed from an individual plant; but it was found that there was no correlation between the size of the seed and the percentage content of oil.

Some varieties of soy beans show a marked tendency to shorten the time required for reaching maturity when planted late in the season, but no correlation was found between the date of planting and the size of the seed or their oil content. These properties appear to be influenced more by the character than by the length of the growing period.

Different varieties of soy beans grown under the same conditions showed marked differences in oil content and very great differences in size of the seed. Although different varieties of cotton showed decided differences in the size of the seed, there was very little difference

in the percentage oil content. The different varieties of soy beans did not respond alike to changes in seasonal conditions.

In tests with several varieties of soy beans grown under a very wide range of conditions there were found differences of more than 100 per cent in the size of the beans and very large differences in oil content. Here, again, the different varieties were not affected alike by changes in the environment. It was not practicable to grow cotton under such diverse conditions, but the difference in oil content of the seed as grown in the Coastal Plain and the Piedmont regions of the South was greater than the varietal differences when grown in the same environment. All varieties respond very much alike to changes in the environment.

Because of the interdependence of soil and climate with respect to temperature and water supply it is difficult or impossible to develop far-reaching generalizations as to the specific effects of either independently of the other on plant development. Six Upland varieties of cotton were grown three consecutive years on adjoining but contrasted soil types in northern Georgia. Each year the clay soil gave heavier seed than the sandy loam, but the relative oil content on the two soil types varied from year to year. In experiments with several varieties of soy beans only small differences were obtained in the size and oil content of the seed grown on these two soil types. Similar results were obtained with peanuts. Field experiments with soy beans and peanuts on sharply contrasted soil types at Arlington Experiment Farm, Va., and vicinity gave more decided differences in size and oil content of the seed. A number of tests with soy beans, peanuts, and sunflower were carried out also on different soil types under controlled conditions, using for the purpose large earthen pots set into the soil. The various tests were carried out under a wide range of soil types and climatic conditions, and the results as a whole emphasize the fact that the relative effects of different soil types are not specific and constant, but depend largely on seasonal conditions.

From the data in hand it is concluded that under practical conditions climate is a more potent factor than soil type in controlling the size of the seed and its oil content, probably because those conditions of the atmosphere which constitute the climate largely control the corresponding conditions of the soil.

Within ordinary limits the relative fertility of the soil appears to be a minor factor in influencing the size of the seed and its oil content. In fertilizer tests with cotton the addition of a complete fertilizer to an unproductive soil gave larger seed and a considerably higher percentage of oil. Applications of nitrogen in increasing quantities did not affect the size of the seed, but lowered the percentage of oil, while increasing applications of phosphorus or potassium did not affect either character.

In pot-culture tests with soy beans the addition of phosphorus did not change the size of the seed, but increased the oil content. Potassium was without decided effect. In similar tests with peanuts neither phosphorus nor potassium affected the oil content.

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PRELIMINARY AND MINOR PAPERS

STUDIES IN THE EXPANSION OF MILK AND CREAM

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[A report on a series of experiments conducted for the Dairy Division, Bureau of Animal Industry]

INTRODUCTION

On May 27, 1913, the Dairy Division of the Bureau of Animal Industry requested the Bureau of Standards to determine the coefficient of expansion of market milk, single cream, and double cream. It was thought that the examination of a few samples of each would be sufficient to serve the purpose. Subsequent observations, however, showed that this was not the case. The wide variation of the rate of expansion of the samples first examined made it apparent that a much greater number of samples would be required than had been anticipated. The results here published are therefore the outgrowth of what was originally expected to be only a very few determinations.

OBJECT OF THE WORK

The principal object in undertaking the work was to determine the change in volume which occurs when the temperature of a given volume of milk or cream is changed and from the rate of change of volume to construct a table of relative volumes of milk and cream at various temperatures. For example, when milk is pasteurized and put into containers at a high temperature, it is sometimes desirable to know what volume of milk must be measured out at that temperature, in order that it may occupy a required volume at some standard temperature.

In the enforcement of the pure food and drugs laws by the Bureau of Chemistry it is held that a container shall have its full nominal capacity at 20° C. (68° F.)—that is, a container labeled as holding 1 gallon must hold 231 cubic inches at 20° C. For the sake of uniformity and also because 20° C. is a reasonable and convenient temperature, it has been chosen as the basis of the table of volumes of milk and cream published herewith. The samples of cream submitted by the Dairy Division were prepared from mixed herd milk produced at the Dairy Division Experiment Farm, Beltsville, Md. The percentage of fat was determined in each case by the Babcock test, and the samples are believed to be normal cream for the stated percentages of fat.[2]

METHOD OF DETERMINATION

The principle employed in determining the rate of expansion was to measure the change of density with change of temperature and from that to calculate the change in volume.

[1] The author would acknowledge his indebtedness to Miss Alice Purinton, formerly of the Bureau of Standards, and to Mr. E. L. Peffer for assistance rendered in the work.

[2] These samples were prepared and the fat determinations made by Mr. R. H. Shaw, of the Dairy Division.

The density determinations were made by what is commonly known as the method of hydrostatic weighing. By this method a sinker or plummet of known mass and volume is suspended in the liquid under examination and weighed. The density of the liquid is then calculated by means of the equation

$$D_t = \frac{W - w\left(1 - \frac{\rho}{S.4}\right)}{V_t}$$

in which D_t = density [1] of the liquid at the temperature t;
W = weight of sinker in vacuo;
w = apparent weight of sinker in liquid;
ρ = air density;
S.4 = assumed density of brass weights;
V_t = volume of sinker at temperature t.

This method of determining densities, though very accurate when used under suitable conditions, is open to criticism when applied to a nonhomogeneous liquid, such as milk or cream. There is, of course, a constant tendency for the fat of the sample under investigation to separate out and rise to the surface and for the heavier components to sink to the bottom. The density of a nonhomogeneous liquid determined by this method will therefore tend to be too low if the sinker is suspended near the surface of the liquid and too high if suspended near the bottom. The difficulty, however, may be largely overcome by the frequent stirring of the sample and still more effectively by the use of a sinker of such a length as to reach nearly from the top to the bottom of the liquid. The average density of the displaced liquid will then be nearly the same as the average density of the whole mass of liquid, and the density determined will be nearly the average density of the sample.

DESCRIPTION OF APPARATUS

The apparatus employed in making the density determinations is described in publications of the Bureau of Standards.[2] Its essential parts are as follows:

The sample to be tested is placed in a tube surrounded by a water bath kept in constant circulation. This bath is in turn surrounded by another, which is also kept in constant circulation. The temperature of the outer bath may be kept constant or varied at will by the adjustment of the energy through an electric heating coil and by the flow of refrigerating brine in a coil, around which the water in the bath may be made to circulate. A sinker of known mass and volume is suspended from one arm of a sensitive balance placed above the other apparatus. The temperature is read from two mercury thermometers placed in water in a second tube similar to that in which the sample is placed, the two tubes being placed side by side within the inner circulating bath. The thermometers are suspended from a movable cover, which may be rotated to bring them successively into position for reading.

[1] Throughout this report the term "density" is used to denote the mass per unit of volume and is expressed in grams per milliliter. The densities are therefore numerically the same as specific gravities as terms of water at 4° C. are used.

[2] Bingham, E. W. Viscosity and thermal expansion of linseed oil and turpentine. U. S. Dept. Com. and Labor, Bur. Standards, Technologic Papers, 1, 17 p., 1907.

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METHOD OF PROCEDURE

The sample of milk to be investigated is placed in the containing tube, the sinker immersed, and the tube placed in position in the temperature-control bath. The temperature is then brought to the point at which the density is first to be measured and is allowed to remain constant until the apparatus reaches a condition of temperature equilibrium. Observations are then begun. First, a weighing is made with the sinker immersed in the sample and suspended from the arm of the balance. Then the temperature is read on each of the two thermometers; next, the sinker is detached and a weighing made with the sinker off, but with the suspension wire still passing through the surface of the liquid. The difference between these weighings is the apparent weight of the sinker in the liquid at the temperature of observation. The object of making the second weighing with the suspension wire still passing through the surface is to eliminate the surface-tension effect on the suspension wire. In order that the suspension wire may be kept straight and in position whether the sinker is attached or not, a small secondary sinker is kept suspended at all times, and the large sinker of known mass and volume is attached to that. When not attached, the large sinker rests on the bottom of the tube and remains standing in an upright position. Immediately after the weighing with the sinker detached, a second weighing is made with it again attached, after which the temperature is again observed on the two thermometers.

The observations at each point therefore consist of two weighings with the sinker attached, one weighing with it detached, and two readings on each of two thermometers. The reason for making two weighings with the sinker attached and only one with it detached is because in the former case a slight change of the temperature of the liquid will make an appreciable change in the weighing, on account of the large volume of the immersed sinker, while in the latter case the change is not appreciable.

After completing the observations at one point, the temperature is changed to the next one in the series and the process is repeated in the same order.

TEMPERATURE RANGE OF DENSITY DETERMINATIONS

At the beginning of the work it was intended to cover the temperature range from 0° to 50° C., but the rate of expansion at low temperatures was found to differ so much from the rate at higher temperatures that the expansion over the entire range could not be expressed by a simple equation. This is especially true of samples having a low fat content. In certain samples a point of maximum density was found at a temperature in the region of 5° C. This is what might be expected—from the similar behavior of water. In the samples containing higher percentages of fat the point of maximum density was not found, but the rate of expansion was noticeably less at the low than at the high temperatures.

The rate of expansion was especially desired at the higher temperatures, and since it was found that the results at temperatures between 20° and 50° C. could be well expressed by a simple equation, it was decided to cover only this temperature range, and when approximate results are desired beyond this range, to extrapolate from the results

over the range covered. The values at the lower temperatures obtained in this way will, of course, not be as near the truth as would be the case if density determinations were made at the low temperatures; but, on the other hand, to include determinations at the lower temperatures would render less accurate the reduced values at the higher temperatures—that is, the assumed equation would not come so near expressing the actual rate of expansion over the temperature range where accuracy is most desired. For that reason in making the "least squares" reduction of the observations at the various temperatures, only the observed densities at 20°, 30°, 40°, and 50° C. were considered.

CALCULATION OF RESULTS

After completing the observations given in Table I at as many points as desired, the density at each point was calculated, and from the density values at the different temperatures the rate of change was determined.

For convenience in calculation it was assumed that the rate of change of density with change of temperature could be expressed with sufficient exactness by means of an equation of the form

$$D_t = D_x + \alpha(t-x) + \beta(t-x)^2 + \gamma(t-x)^3 +$$

in which D_t = the density at any temperature t,
D_x = the density at some standard temperature x,
α, β, and γ = constants to be determined for each sample investigated.

In practice it was found that for certain temperature ranges the expansion was represented within the limits of experimental error, by the above equation, with all terms above the second power omitted. By means of a "least squares" method the observations on each sample given in Table I have been reduced and the calculated values of Dx, α, and β are given in Table IV. Observations and calculations of density for an average sample of cream are given in Table III. It will be seen from the closeness of the agreement between the calculated and the observed values of the density at various temperatures that the assumed equation comes very near expressing the actual rate of expansion of the different samples at the time the density determinations were made.

TABLE I.—*Observed densities of milk and cream*

Date.	Fat content.[a]	Temperature.	Density.	Date.	Fat content.	Temperature.	Density.
1913.	*Per cent.*	*° C.*	*G./c. c.*	1913.	*Per cent.*	*° C.*	*G./c. c.*
July 12.....	0.025	0	1.0381	June 3.....	3.5	0	1.0362
	.025	10	1.0368		3.5	10	1.0348
	.025	20	1.0356		3.5	20	1.0320
	.025	30	1.0322		3.5	30	1.0284
	.025	40	1.0284				
	.025	50	1.0236				
				June 7.....	3.5	20	1.0311
July 11.....	2.5	0	1.0348		3.5	30	1.0290
	2.5	5	1.0343		3.5	40	1.0237
	2.5	10	1.0348				
	2.5	20	1.0317				
	2.5	30	1.0292	Nov. 19.....	3.5	20	1.0314
	2.5	40	1.0258		3.5	30	1.0279
	2.5	50	1.0212		3.5	40	1.0238
					3.5	50	1.0191

[a] The percentages of fat here given are as reported by the Bureau of Animal Industry at the time the samples were prepared.

TABLE I.—*Observed densities of milk and cream*—Continued

Date.	Fat content.	Temperature.	Density.
1913.	*Per cent.*	*° C.*	*G./c. c.*
July 10	5	0	1. 0344
	5	5	1. 0357
	5	10	1. 0353
	5	20	1. 0322
	5	30	1. 0299
	5	40	1. 0217
	5	50	1. 0171
Aug. 26	5	20	1. 0268
	5	30	1. 0235
	5	40	1. 0192
	5	50	1. 0144
July 9	7. 5	0	1. 0327
	7. 5	5	1. 0316
	7. 5	10	1. 0316
	7. 5	20	1. 0274
	7. 5	30	1. 0246
	7. 5	40	1. 0209
	7. 5	50	1. 0159
Nov. 19	7. 5	20	1. 0261
	7. 5	30	1. 0226
	7. 5	40	1. 0184
	7. 5	50	1. 0136
July 8	10	0	1. 0304
	10	5	1. 0310
	10	10	1. 0295
	10	20	1. 0242
	10	30	1. 0197
	10	40	1. 0152
	10	50	1. 0106
July 2	15	0	1. 0256
	15	10	1. 0216
	15	20	1. 0170
	15	30	1. 0114
	15	40	1. 0061
	15	50	1. 0028
Aug. 25	15	20	1. 0161
Aug. 26	15	30	1. 0112
	15	40	1. 0061
	15	50	1. 0011
June 3	20	0	1. 0242
	20	10	1. 0208
	20	20	1. 0137
June 7	20	20	1. 0141
	20	30	1. 0081
	20	40	1. 0018
July 3	20	0	1. 0214
	20	10	1. 0169
	20	20	1. 0106
	20	30	1. 0052
	20	40	. 9995
	20	50	. 9950

Date.	Fat content.	Temperature.	Density.
1913.	*Per cent.*	*° C.*	*G./c. c.*
June 27	25	10	1. 0136
	25	20	1. 0070
	25	30	1. 0007
	25	40	. 9944
	25	50	. 9890
Nov. 20	25	20	1. 0107
	25	30	1. 0028
	25	40	. 9965
	25	50	. 9911
June 26	30	10	1. 0108
	30	20	. 9997
	30	30	. 9940
	30	40	. 9884
	30	50	. 9827
Aug. 23	30	20	. 9966
	30	30	. 9918
	30	40	. 9864
	30	50	. 9813
Nov. 20	30	20	. 9978
	30	30	. 9933
	30	40	. 9886
	30	50	. 9832
Nov. 21	30	20	1. 0044
	30	30	. 9960
	30	40	. 9896
	30	50	. 9842
June 26	35	0	1. 0129
	35	10	1. 0057
	35	20	. 9970
	35	30	. 9879
	35	40	. 9807
	35	50	. 9750
June 3	40	0	1. 0088
	40	10	1. 0037
	40	20	. 9931
	40	30	. 9838
June 7	40	20	. 9940
	40	30	. 9845
	40	40	. 9763
June 25	40	0	1. 0050
	40	5	1. 0036
	40	10	1. 0027
	40	20	. 9931
Aug. 26	40	30	. 9837
	40	40	. 9765
	40	50	. 9710

CALCULATION OF RELATIVE VOLUMES

After having determined the density of the several samples of milk and cream at the various temperatures, the observations for each sample were reduced by the method of least squares, as already stated, and the value of $D_{35°}$, α, and β determined for each sample. The values are shown in Table IV. The method of reducing the observations to obtain constants in the assumed equations—namely, values $D_{35°}$, α, and β—is as follows:

$D_t = D_{35} + \alpha(t-35) + \beta(t-35)^2.$

$(C_1 = t - t_m;\ C_2 = C_1^2 - (C_1^2)_m.\ N = D_t - (D_t)_m.)$

$(\Sigma C_1^2\alpha + \Sigma C_{12}\beta C = \Sigma C_1 N.)$

$\Sigma C_1 C_2\alpha + \Sigma C_2^2\beta = \Sigma C_2 N.$

$500\alpha + 0 = -0.1990.$

$0 + 40{,}000\,\beta = -0.140.$

$\alpha = -0.000398.$

$\beta = -0.0000035.$

$$X_m + \frac{\Sigma C_1^2}{n}\beta = D_m.$$

X_m = density at mean temperature = $D_{35°}$.

D_m = mean of densities.

n = number of observations = 4.

$X_m = D_{35°} = D_m - 125B.$

$D_{35°} = 1.02995 + 0.00004_4 = 1.03039.$

$D_t = D_{35°} + \alpha(t-35) + \beta(t-35)^2.$

$D_t = 1.03039 - 0.000398(t-35) - 0.0000035(t-35)^2.$

Calculation for Dt

t	0.025 per cent butter fat (skim milk).								
	C_1	C_1^2	C_2	C_1C_2	C_2^2	Dt	N	C_1N	C_2N
°C.									
20	−15	225	+100	−1.500	10.000	1.0356	+0.00565	−0.08475	+0.505
30	− 5	25	−100	+ 500	10.000	1.0322	+ .00225	− .01125	− .225
40	+ 5	25	−100	− 500	10.000	1.0284	− .00155	− .00775	+ .155
50	+15	225	+100	+1.500	10.000	1.0236	− .00635	− .09525	− .635
35		4)500		0.000	40.000	1.02996		− .10000	− .140
		125							

t	0.025 per cent butter fat (skim milk).						
	$t-35$	$(t-35)^2$	$\alpha(t-35)$	$\beta(t-35)^2$	Dt (observed)	Dt (calculated.)	Obs.—cal.
20	−15	225	+0.00597	−0.00079	1.0356	1.0356	0
30	− 5	25	+ .00189	− .00009	1.0322	1.0322	0
40	+ 5	25	− .00189	− .00009	1.0284	1.0284	0
50	+15	225	− .00597	− .00079	1.0236	1.0236	0

TABLE II.—*Densities of milk and cream corresponding to various percentages of fat*

Percentage of fat.	$D_{\frac{20}{20}}°$	$D_{\frac{20}{4}}°$	$D_{\frac{34}{4}}°$	α	β
	Sp. gr.	*Sp. gr.*	*Sp. gr.*		
0. 025	1. 037	1. 035	1. 030	−0. 00040	−0. 000005
1	1. 036	1. 034	1. 029	. 00040	. 000004
2	1. 035	1. 033	1. 028	. 00040	. 000004
3	1. 034	1. 032	1. 027	. 00041	. 000004
4	1. 032	1. 031	1. 025	. 00041	. 000003
5	1. 031	1. 029	1. 024	. 00041	. 000003
6	1. 030	1. 028	1. 022	. 00042	. 000003
7	1. 029	1. 027	1. 021	. 00042	. 000002
8	1. 027	1. 026	1. 020	. 00043	. 000002
9	1. 026	1. 024	1. 018	. 00044	. 000001
10	1. 025	1. 023	1. 016	. 00045	. 000001
11	1. 024	1. 022	1. 015	. 00046	. 000000
12	1. 022	1. 020	1. 013	. 00047	. 000000
13	1. 020	1. 019	1. 012	. 00048	. 000000
14	1. 019	1. 017	1. 010	. 00048	+. 000001
15	1. 018	1. 016	1. 009	. 00049	. 000001
16	1. 017	1. 015	1. 007	. 00050	. 000001
17	1. 016	1. 014	1. 006	. 00051	. 000002
18	1. 015	1. 013	1. 005	. 00052	. 000002
19	1. 014	1. 012	1. 004	. 00052	. 000002
20	1. 013	1. 011	1. 003	. 00053	. 000003
21	1. 012	1. 010	1. 001	. 00054	. 000003
22	1. 011	1. 009	1. 000	. 00055	. 000003
23	1. 010	1. 008	. 999	. 00057	. 000004
24	1. 009	1. 007	. 998	. 00058	. 000004
25	1. 008	1. 007	. 997	. 00059	. 000004
26	1. 008	1. 006	. 996	. 00061	. 000005
27	1. 007	1. 005	. 994	. 00062	. 000005
28	1. 006	1. 004	. 993	. 00063	. 000005
29	1. 005	1. 003	. 992	. 00064	. 000005
30	1. 004	1. 002	. 991	. 00065	. 000006
31	1. 003	1. 001	. 990	. 00066	. 000006
32	1. 002	1. 000	. 989	. 00067	. 000007
33	1. 001	. 999	. 988	. 00068	. 000007
34	1. 000	. 998	. 986	. 00069	. 000008
35	. 999	. 998	. 985	. 00071	. 000008
36	. 999	. 997	. 984	. 00072	. 000008
37	. 998	. 996	. 983	. 00073	. 000009
38	. 997	. 995	. 982	. 00074	. 000009
39	. 996	. 994	. 981	. 00075	. 000009
40	. 995	. 993	. 980	. 00076	. 000010

TABLE III.—*Sample set of observations—cream containing 25 per cent of fat*

[Samples received Nov. 19, 1913; observations made Nov. 20, 1913]

Temperature.				Balance readings.	Apparent weight.	Air buoyancy.[a]	Corrected weight.	Weight of sinker in vacuo.	Displaced liquid weight.	Volume.	Density of liquid at observed temperature.	Correction to reduce to integral temperature.	Density of liquid reduced to integral degrees.	Integral temperature.
Observed.		Corrected.												
°C.	°C	°C.	°C.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	C. c.				°C.
[b] No. 2040:	[b] No. 4653:					0.00119								
0	[c] + 0.04													
20.50	20.48	20.50	20.52	31.7734	51.7631									
20.56	20.50	20.56	20.54	83.5365	51.7640									
Average		20.53		31.7725	51.7636	−.0073	51.7563	99.9630	48.2067	47.7135	1.01034	+0.00040	1.01074	20
0	0													
30.20	30.22	30.20	30.22	31.3950	52.1200									
30.20	30.21	30.20	30.21	83.5150	52.1215									
Average		30.21		31.3935	52.1208	−.0073	52.1135	99.9630	47.8495	47.7242	1.00262	+ .00014	1.00276	30
[b] No. 264:														
[c] −0.03	[c] +0.07													
39.84	39.80	39.81	39.87	31.1096	52.4012									
39.86	39.78	39.83	39.85	83.5108	52.3962									
Average		39.84		31.1146	52.3987	−.0074	52.3913	99.9630	47.5717	47.7350	.99658	− .00009	.99649	40
[b] No. 2036:	[b] No. 8998:													
[c] −0.03	[c] −0.30													
50.04	50.32	50.01	50.02	30.8460	52.6540									
50.12	50.38	50.09	50.08	83.5000	52.6480									
Average		50.05		30.8520	52.6510	−.0074	52.6436	99.9630	47.3194	47.7467	.99105	+ .00003	.99108	50

[a] In the column headed "Air buoyancy," 0.00119 is the air density and 0.0073 gm. is the buoyancy correction to be applied to the apparent weight.
[b] Number of the thermometer used.
[c] Corrections for temperature.

TABLE IV.—*Observed and calculated densities of milk and cream at different temperatures and with different percentages of fat*[a]

Fat content.	$D_{35}°$	α	β	Temperature.	Dt. Observed.	Dt. Calculated.	Observed minus calculated.
Per cent.							
0.025 ..	1.03039	−0.00040	−0.000004	20	1.0356	1.0356	0
				30	1.0322	1.0322	0
				40	1.0284	1.0284	0
				50	1.0236	1.0236	0
2.5	1.0276	− .00035	− .000005	20	1.0317	1.0317	0
				30	1.0292	1.0292	0
				40	1.0258	1.0258	0
				50	1.0212	1.0212	0
3.5	1.0259	− .00041	− .000003	20	1.0314	1.0314	0
				30	1.0279	1.0279	0
				40	1.0238	1.0238	0
				50	1.0191	1.0191	0
5	1.0259	− .00053	− .000006	20	1.0322	1.0327	[b]− 5
				30	1.0299	1.0285	+14
				40	1.0217	1.0231	−14
				50	1.0171	1.0166	+ 5
5	1.0214	− .00042	− .000004	20	1.0268	1.0268	0
				30	1.0235	1.0234	+ 1
				40	1.0192	1.0193	− 1
				50	1.0144	1.0144	0
7.5	1.0229	− .00038	− .000005	20	1.0274	1.0274	0
				30	1.0246	1.0247	− 1
				40	1.0209	1.0208	+ 1
				50	1.0159	1.0159	0
7.5	1.0206	− .00042	− .000003	20	1.0261	1.0261	0
				30	1.0226	1.0226	0
				40	1.0184	1.0184	0
				50	1.0136	1.0136	0
10	1.0174	− .00045	0	20	1.0242	1.0242	0
				30	1.0197	1.0197	0
				40	1.0152	1.0152	0
				50	1.0106	1.0106	0
15	1.0086	− .00048	+ .000006	20	1.0170	1.0171	− 1
				30	1.0114	1.0111	+ 3
				40	1.0061	1.0063	− 2
				50	1.0028	1.0027	+ 1
15	1.0086	− .00050	0	20	1.0161	1.0161	0
				30	1.0112	1.0111	+ 1
				40	1.0061	1.0061	0
				50	1.0011	1.0011	0
20	1.0023	− .00052	+ .000002	20	1.0106	1.0107	− 1
				30	1.0052	1.0050	+ 2
				40	.9995	.9997	− 2
				50	.9950	.9949	+ 1

[a] The temperatures from which the reductions are made are the same for all samples—namely, 20°, 30°, 40°, and 50° C.—and for that reason C_1, C_2, etc., will be the same in all cases.

[b] The lack of agreement between the observed and the calculated values of density indicates that one or more of the observed values are considerably in error. All determinations on this sample should be discarded.

TABLE IV.—*Observed and calculated densities of milk and cream at different temperatures and with different percentages of fat*—Continued

Fat content.	$D_{20}°$	α	β	Temperature.	D_t. Observed.	D_t. Calculated.	Observed minus calculated.
Per cent.							
25.....	0.9075	−0.00000	+0.000002	20	1.0070	1.0070	0
				30	1.0007	1.0006	+1
				40	0.9944	0.9945	−
				50	0.9890	0.9890	0
25.....	.9095	− .00065	+ .000006	20	1.0107	1.0107	0
				30	1.0028	1.0029	−1
				40	0.9965	0.9964	+1
				50	0.9911	0.9911	0
30.....	.9912	− .00057	0	20	0.9997	0.9997	0
				30	0.9940	0.9940	0
				40	0.9884	0.9884	0
				50	0.9827	0.9827	0
30.....	.9891	− .00051	− .000001	20	0.9966	0.9966	0
				30	0.9918	0.9917	+1
				40	0.9854	0.9865	−1
				50	0.9813	0.9812	+1
30.....	.9910	− .00049	− .000002	20	0.9978	0.9978	0
				30	0.9933	0.9934	−1
				40	0.9880	0.9885	+1
				50	0.9832	0.9832	0
30.....	.9926	− .00067	0	20	1.0044	1.0044	0
				30	0.9960	0.9962	−2
				40	0.9896	0.9894	+2
				50	0.9842	0.9842	0
35.....	.9841	− .00073	− .000008	20	0.9970	0.9970	0
				30	0.9879	0.9880	−1
				40	0.9807	0.9806	+1
				50	0.9750	0.9750	0
40.....	.9799	− .00074	+ .000010	20	0.9931	0.9931	0
				30	0.9837	0.9838	−1
				40	0.9765	0.9764	+1
				50	0.9710	0.9710	0

These results were then plotted on coordinate paper of such a size that densities could be plotted and read to one in the fourth place, and α and β to one in the fifth and sixth places, respectively. From these curves the values of α and β for various densities of milk and cream are tabulated in Table II. The curves are shown on a reduced scale in figure 1.

In figure 2 is shown the relation between the density of the samples and the percentage of butter fat contained in them.

Having determined the density of each sample and the rate of change of density with change of temperature, it was possible to calculate the volume of any sample at any temperature in terms of the volume at any other temperature within the limits covered.

It was suggested by the Dairy Division of the Bureau of Animal Industry that 20° C. (68° F.) be chosen as the standard temperature and

that the relative volumes at other temperatures be given on the basis of unit volume at this temperature. It was also suggested that for the

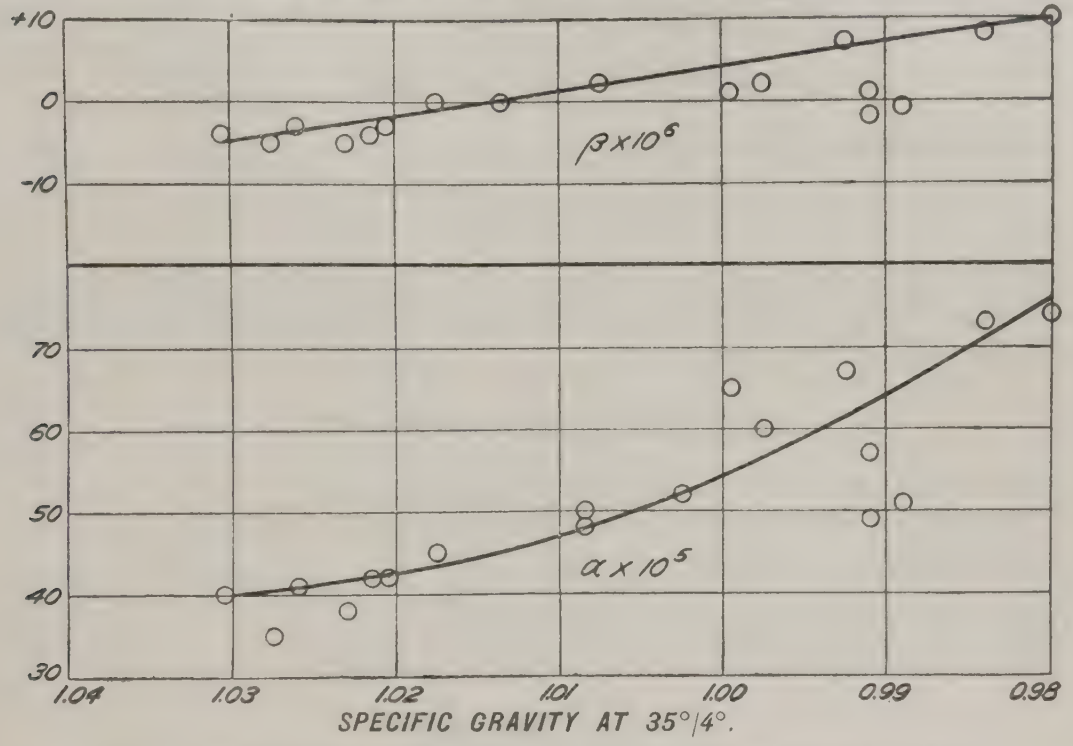

FIG. 1.—Specific gravity of milk and cream at 35°/4° C., showing values of α and β[1].

convenience of those by whom the table would be used the temperatures be given on the Fahrenheit scale, and that the densities at 20° C. in grams per cubic centimeter be changed to specific gravities at 20° C. in terms of

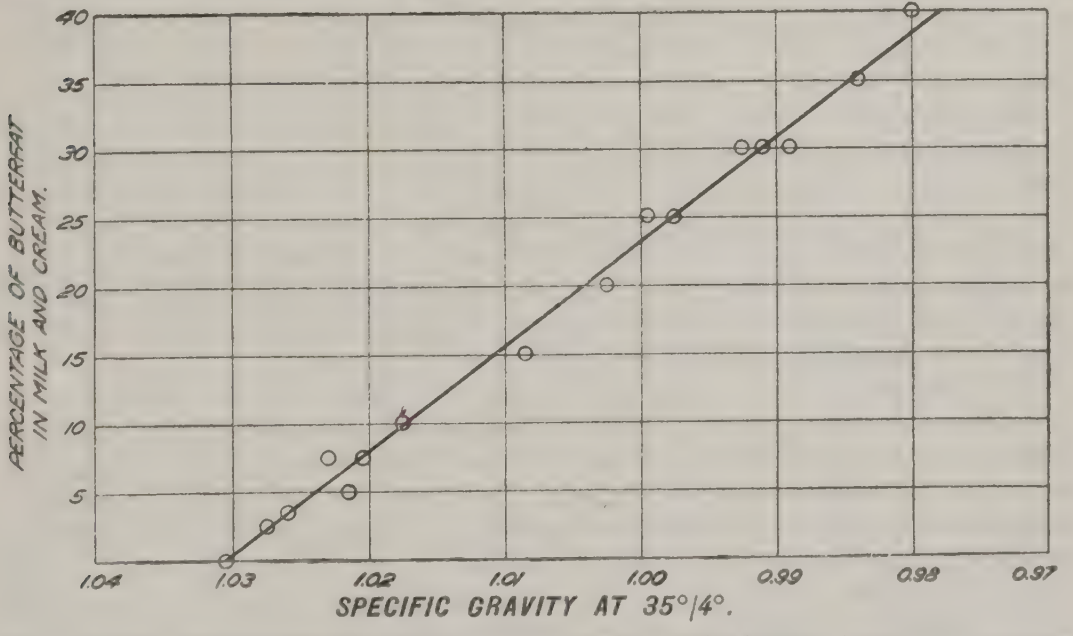

FIG. 2.—Specific gravity of milk and cream at 35°/4° C., showing relation between density and percentage of butter fat.

water at that temperature as unity. These changes have accordingly been made.

[1] The specific gravity at 35°/4° C. means the specific gravity at 35° C. in terms of water at 4° C. as unity. This is numerically the same as the density at 35° C. in gms. per c. c.

It was found that the calculation of volumes could be most conveniently accomplished by changing the basis of the calculation from 35° to 20° C. The equation

$$D_t = D_{35}° + \alpha(t-35) + \beta(t-35)^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 1$$

was accordingly transformed to

$$D_t = D_{20}° + \alpha^1(t-20) + \beta^1(t-20)^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 2$$

and the values of α^1 and β^1 determined. It can be shown that $\alpha^1 = \alpha - 30\beta$ and that $\beta^1 = \beta$. As a further convenience in calculation, the equation was changed to the form

$$V_t = V_{20}° \left[1 + A(t-20) + B(t-20)^2\right] \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 3$$

in which A and B may be found in terms of α^1 and β^1.

It would, of course, be possible to calculate the volumes directly by means of equation 2, since $d = \frac{m}{V}$ or $V = \frac{m}{d}$, but the calculation is much more easily done by means of equation 3.

The volumes thus calculated are given in Table V.[1]

SOURCES OF ERROR

It has already been pointed out that one source of error is the gradual separation of the sample under investigation into its constituent parts, the fat rising to the top and the heavier portions settling toward the bottom. The greatest source of error, however, is probably in the assumed percentage of fat in the sample at the time the density determinations are made. This will be explained somewhat in detail. The samples of milk and cream were generally prepared at the Bureau of Animal Industry in the morning and brought to the Bureau of Standards in the afternoon of the same day. The density determinations were made on the following day. During this interval of time between the preparation of the samples and the making of the density determinations the samples could be kept sweet without difficulty, but there was in many cases a considerable amount of separation and "churning" of the fat, so that granules of butter were collected on the neck and the cap of the bottle in which the sample had been kept.

This change in the percentage of fat contained in the sample was, of course, always in such a direction that the sample at the time its density was determined contained a lower percentage of fat than that reported by the Bureau of Animal Industry at the time the sample was prepared. For that reason, since the density increases with decreasing percentage of fat, the densities of the different samples will in most cases be somewhat too large for the percentages of fat to which they are intended to correspond; or, in other words, the tabulated percentage of fat in a given sample is somewhat too high. In the tabulated values of percentage of fat and corresponding density (Table II), the density

[1] These data also appear as Table II, U. S. Dept. Agr. Bul. 98, p. 6–9.

is in each case given as corresponding to the percentage of fat in the sample at the time it was prepared. In reality it corresponds, not to that percentage of fat, but to the percentage in the sample at the time the density was determined. No attempt will be made at the present time to estimate how great this discrepancy may be; in some cases it is quite appreciable.

Other sources of error are the temperature observations and the weighings of the sinker. The weighings were made to tenths of a milligram and were probably correct in most cases to about half a mg. Errors greater than 1 or 2 mg. would be unlikely to occur. The thermometers were graduated to tenths of a degree centigrade and were read to hundredths. The mean of the four readings taken at each temperature was probably correct within one or two hundredths of a degree. Errors of more than three hundredths would not be expected. If both of these maximum errors should occur in the same set of observations and both should be in the same direction, the resulting error in the density would be about six units in the fifth decimal place. Even such an error would not be serious in the present instance, as the density values are used in the table only to the fourth place. The density determinations are almost certainly accurate to that degree. In the calculation of the densities the results were carried to the fifth place, and they are seen to be concordant in most cases to somewhat better than one in the fourth place.

CONCLUSION

Examination of the results here presented (see Table III) shows that for the individual samples examined the density determinations may be depended upon to about one unit of the fourth decimal place. These values, however, when plotted (see figs. 1 and 2), present certain irregularities which are far too great to be accounted for by errors in the determinations. For example, four different samples were examined, each of which was supposed to contain 30 per cent of fat. The densities of the four samples at 35° C. were found to be in satisfactory agreement, and for each sample the agreement between the observed and calculated densities at other temperatures was such as to throw no suspicion upon the determinations; and yet the rate of expansion of the four samples was widely different. Only one out of the four fitted reasonably well into the series formed by the samples above and below 30 per cent. This and similar anomalies for certain other samples make it appear that the rate of expansion of any given sample depends upon something more than the density or the percentage of fat present. It undoubtedly depends upon the physical and chemical condition of the sample at the time the observations are made. This condition is probably largely dependent upon the time that has elapsed since the preparation of the sample and upon the temperature at which it has been kept. That being the case, it would probably be impossible to find any fixed relation that would express accurately the rate of expansion of all percentages of butter fat under all conditions. Further investigation to determine the effect of time and temperature upon the rate of expansion would be of considerable interest, and such an investigation of these and similar points will be necessary before the rate of expansion under all ordinary conditions can be accurately known.

Although these results can not be considered as final, it is believed that for the purpose for which this work was undertaken results have been obtained which are of sufficient accuracy. It is, however, desirable that further work be done by a method better adapted to the nature of the liquid investigated. In future work greater precaution should be taken to prevent the fat from being removed from the samples before the density determinations are made, and it would be very desirable if the percentage of fat in each sample could be redetermined after the density determinations have been made. As these results were obtained with mixed milk it would also be desirable to compare the density of milk in different breeds of cows and the variation within the breeds.

TABLE V.—*Volume*[a] *of milk and cream at various temperatures occupied by unit volume at 68° F. (20° C.)*

Percentage of butterfat.	Temperature (° F.).											
	50	52	54	56	58	60	62	64	66	68	70	72
	Volume.											
0.025	0.9980	0.9980	0.9985	0.9985	0.9990	0.9990	0.9990	0.9995	0.9995	1.0000	1.0000	1.0005
1	9980	.9980	.9985	.9985	.9990	.9990	.9990	.9995	.9995	1.0000	1.0000	1.0005
2	.9975	.9975	.9980	.9980	.9985	.9990	.9990	.9995	.9995	1.0000	1.0000	1.0005
3	.9975	.9975	.9980	.9980	.9985	.9990	.9990	.9995	.9995	1.0000	1.0000	1.0005
4	.9975	.9975	.9980	.9980	.9985	.9985	.9990	.9995	.9995	1.0000	1.0000	1.0005
5	.9975	.9975	.9980	.9980	.9985	.9985	.9990	.9995	.9995	1.0000	1.0000	1.0005
6	.9970	.9975	.9975	.9980	.9980	.9985	.9990	.9995	.9995	1.0000	1.0000	1.0005
7	.9970	.9970	.9975	.9975	.9980	.9985	.9985	.9990	.9995	1.0000	1.0000	1.0010
8	.9970	.9970	.9975	.9975	.9980	.9985	.9985	.9990	.9995	1.0000	1.0005	1.0010
9	.9965	.9965	.9970	.9975	.9980	.9985	.9985	.9990	.9995	1.0000	1.0005	1.0010
10	.9965	.9965	.9970	.9975	.9980	.9985	.9985	.9990	.9995	1.0000	1.0005	1.0010
11	.9965	.9965	.9970	.9975	.9980	.9985	.9985	.9990	.9995	1.0000	1.0005	1.0010
12	.9955	.9960	.9965	.9970	.9975	.9980	.9985	.9990	.9990	1.0000	1.0005	1.0010
13	.9955	.9960	.9965	.9970	.9975	.9980	.9985	.9990	.9990	1.0000	1.0005	1.0010
14	.9950	.9955	.9960	.9970	.9975	.9980	.9985	.9990	.9990	1.0000	1.0005	1.0010
15	.9950	.9955	.9960	.9970	.9975	.9980	.9985	.9990	.9990	1.0000	1.0005	1.0010
16	.9950	.9955	.9955	.9965	.9970	.9980	.9985	.9990	.9990	1.0000	1.0005	1.0010
17	.9945	.9950	.9955	.9965	.9970	.9980	.9985	.9990	.9990	1.0000	1.0005	1.0010
18	.9940	.9945	.9950	.9960	.9970	.9980	.9980	.9985	.9990	1.0000	1.0005	1.0010
19	.9940	.9945	.9950	.9960	.9970	.9975	.9980	.9985	.9990	1.0000	1.0005	1.0010
20	.9930	.9940	.9945	.9955	.9965	.9975	.9980	.9985	.9990	1.0000	1.0005	1.0010
21	.9930	.9940	.9945	.9955	.9965	.9975	.9980	.9985	.9990	1.0000	1.0005	1.0010
22	.9930	.9940	.9945	.9955	.9965	.9975	.9980	.9985	.9990	1.0000	1.0010	1.0015
23	.9930	.9940	.9940	.9955	.9965	.9975	.9980	.9985	.9990	1.0000	1.0010	1.0015
24	.9925	.9930	.9940	.9950	.9960	.9975	.9975	.9985	.9990	1.0000	1.0010	1.0015
25	.9925	.9930	.9940	.9950	.9960	.9970	.9975	.9985	.9990	1.0000	1.0010	1.0015
26	.9925	.9930	.9940	.9950	.9960	.9970	.9975	.9985	.9990	1.0000	1.0010	1.0015
27	.9925	.9930	.9940	.9950	.9960	.9970	.9975	.9985	.9990	1.0000	1.0010	1.0015
28	.9915	.9925	.9935	.9945	.9955	.9965	.9975	.9980	.9990	1.0000	1.0010	1.0015
29	.9915	.9925	.9935	.9945	.9955	.9965	.9975	.9980	.9990	1.0000	1.0010	1.0015
30	.9915	.9925	.9935	.9945	.9955	.9965	.9970	.9980	.9990	1.0000	1.0010	1.0020
31	.9915	.9925	.9935	.9945	.9955	.9965	.9970	.9980	.9990	1.0000	1.0010	1.0020
32	.9910	.9920	.9930	.9940	.9950	.9960	.9970	.9980	.9990	1.0000	1.0010	1.0020
33	.9910	.9920	.9930	.9940	.9950	.9960	.9970	.9980	.9990	1.0000	1.0010	1.0020
34	.9910	.9915	.9925	.9940	.9950	.9960	.9970	.9980	.9990	1.0000	1.0010	1.0020
35	.9900	.9915	.9925	.9940	.9940	.9960	.9970	.9980	.9990	1.0000	1.0010	1.0020
36	.9900	.9910	.9920	.9930	.9940	.9955	.9965	.9980	.9990	1.0000	1.0010	1.0025
37	.9890	.9910	.9920	.9930	.9940	.9955	.9965	.9980	.9990	1.0000	1.0010	1.0025
38	.9890	.9910	.9920	.9930	.9940	.9955	.9965	.9980	.9990	1.0000	1.0010	1.0025
39	.9890	.9900	.9915	.9925	.9940	.9955	.9965	.9975	.9990	1.0000	1.0010	1.0025
40	.9890	.9900	.9915	.9925	.9940	.9950	.9960	.9975	.9990	1.0000	1.0010	1.0025

[a] The tabulated values are given to the nearest 0.0005.

TABLE V.—*Volume of milk and cream at various temperatures occupied by unit volume at 68° F. (20° C.)*—Continued

Percentage of butterfat.	Temperature (° F.).											
	74	76	78	80	82	84	86	88	90	92	94	96
	Volume.											
0.025	1.0005	1.0010	1.0010	1.0015	1.0020	1.0025	1.0030	1.0030	1.0035	1.0040	1.0045	1.0050
1	1.0005	1.0010	1.0010	1.0015	1.0020	1.0025	1.0030	1.0030	1.0035	1.0040	1.0045	1.0050
2	1.0010	1.0010	1.0015	1.0020	1.0020	1.0025	1.0030	1.0035	1.0040	1.0040	1.0045	1.0050
3	1.0010	1.0010	1.0015	1.0020	1.0020	1.0025	1.0030	1.0035	1.0040	1.0045	1.0045	1.0055
4	1.0010	1.0010	1.0015	1.0020	1.0020	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055
5	1.0010	1.0015	1.0020	1.0020	1.0025	1.0030	1.0035	1.0035	1.0045	1.0045	1.0050	1.0055
6	1.0010	1.0015	1.0020	1.0020	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0050	1.0060
7	1.0010	1.0015	1.0020	1.0025	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055	1.0060
8	1.0010	1.0015	1.0020	1.0025	1.0030	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055	1.0060
9	1.0010	1.0015	1.0020	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055	1.0060	1.0065
10	1.0015	1.0020	1.0025	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055	1.0060	1.0065
11	1.0015	1.0020	1.0025	1.0025	1.0030	1.0035	1.0040	1.0045	1.0055	1.0055	1.0065	1.0070
12	1.0015	1.0020	1.0025	1.0030	1.0030	1.0035	1.0040	1.0050	1.0055	1.0060	1.0065	1.0070
13	1.0015	1.0020	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055	1.0060	1.0065	1.0070
14	1.0015	1.0020	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055	1.0065	1.0070	1.0075
15	1.0015	1.0025	1.0030	1.0030	1.0035	1.0040	1.0045	1.0055	1.0060	1.0065	1.0070	1.0075
16	1.0015	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055	1.0060	1.0070	1.0075	1.0080
17	1.0015	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0060	1.0060	1.0070	1.0075	1.0080
18	1.0020	1.0025	1.0030	1.0035	1.0040	1.0045	1.0055	1.0060	1.0065	1.0075	1.0080	1.0085
19	1.0020	1.0025	1.0030	1.0035	1.0045	1.0045	1.0055	1.0060	1.0065	1.0075	1.0080	1.0085
20	1.0020	1.0025	1.0030	1.0035	1.0045	1.0050	1.0055	1.0060	1.0070	1.0075	1.0085	1.0090
21	1.0020	1.0025	1.0030	1.0040	1.0045	1.0050	1.0060	1.0065	1.0070	1.0080	1.0085	1.0090
22	1.0020	1.0030	1.0035	1.0040	1.0050	1.0055	1.0060	1.0065	1.0075	1.0080	1.0090	1.0095
23	1.0020	1.0030	1.0035	1.0040	1.0050	1.0055	1.0065	1.0070	1.0075	1.0085	1.0090	1.0095
24	1.0020	1.0030	1.0035	1.0040	1.0050	1.0060	1.0065	1.0070	1.0080	1.0085	1.0095	1.0100
25	1.0020	1.0030	1.0035	1.0045	1.0055	1.0060	1.0070	1.0075	1.0080	1.0090	1.0095	1.0105
26	1.0025	1.0030	1.0040	1.0045	1.0055	1.0060	1.0070	1.0080	1.0085	1.0090	1.0100	1.0110
27	1.0025	1.0030	1.0040	1.0045	1.0055	1.0060	1.0070	1.0080	1.0085	1.0095	1.0100	1.0110
28	1.0025	1.0030	1.0040	1.0045	1.0055	1.0065	1.0075	1.0080	1.0090	1.0095	1.0105	1.0115
29	1.0025	1.0030	1.0040	1.0050	1.0060	1.0065	1.0075	1.0080	1.0090	1.0095	1.0105	1.0115
30	1.0025	1.0035	1.0045	1.0050	1.0060	1.0065	1.0080	1.0085	1.0095	1.0100	1.0110	1.0120
31	1.0025	1.0035	1.0045	1.0050	1.0060	1.0065	1.0080	1.0085	1.0095	1.0100	1.0110	1.0120
32	1.0030	1.0035	1.0045	1.0055	1.0065	1.0070	1.0085	1.0090	1.0100	1.0105	1.0115	1.0125
33	1.0030	1.0035	1.0045	1.0055	1.0065	1.0070	1.0085	1.0090	1.0100	1.0105	1.0115	1.0125
34	1.0030	1.0040	1.0050	1.0055	1.0065	1.0075	1.0085	1.0095	1.0105	1.0110	1.0120	1.0130
35	1.0030	1.0040	1.0050	1.0060	1.0070	1.0075	1.0090	1.0095	1.0105	1.0110	1.0120	1.0130
36	1.0035	1.0045	1.0055	1.0060	1.0070	1.0080	1.0090	1.0100	1.0110	1.0115	1.0125	1.0135
37	1.0035	1.0045	1.0055	1.0060	1.0070	1.0080	1.0095	1.0100	1.0110	1.0115	1.0125	1.0135
38	1.0035	1.0045	1.0055	1.0065	1.0075	1.0085	1.0095	1.0100	1.0115	1.0120	1.0130	1.0140
39	1.0035	1.0045	1.0055	1.0065	1.0075	1.0085	1.0095	1.0105	1.0115	1.0120	1.0130	1.0140
40	1.0035	1.0045	1.0055	1.0065	1.0075	1.0085	1.0095	1.0105	1.0115	1.0125	1.0130	1.0145

TABLE V.—*Volume of milk and cream at various temperatures occupied by unit volume at 68° F. (20° C.)*—Continued

Percentage of butter fat.	Temperature (° F.).										
	98	100	102	104	106	108	110	112	114	116	118
	Volume.										
0.025	1.0055	1.0060	1.0065	1.0070	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0105
1	1.0055	1.0060	1.0065	1.0070	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0105
2	1.0055	1.0060	1.0065	1.0070	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0110
3	1.0060	1.0065	1.0065	1.0070	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0110
4	1.0060	1.0065	1.0065	1.0070	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0110
5	1.0060	1.0065	1.0070	1.0075	1.0080	1.0085	1.0085	1.0090	1.0095	1.0100	1.0110
6	1.0060	1.0065	1.0070	1.0075	1.0080	1.0085	1.0090	1.0090	1.0095	1.0100	1.0110
7	1.0065	1.0070	1.0075	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0105	1.0115
8	1.0065	1.0070	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0105	1.0110	1.0115
9	1.0065	1.0070	1.0080	1.0080	1.0085	1.0090	1.0095	1.0100	1.0105	1.0110	1.0115
10	1.0070	1.0075	1.0080	1.0085	1.0090	1.0090	1.0095	1.0100	1.0105	1.0110	1.0115
11	1.0070	1.0075	1.0080	1.0085	1.0090	1.0095	1.0095	1.0100	1.0105	1.0110	1.0115
12	1.0075	1.0080	1.0085	1.0090	1.0095	1.0095	1.0105	1.0110	1.0115	1.0120	1.0125
13	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0105	1.0110	1.0115	1.0120	1.0125
14	1.0080	1.0085	1.0090	1.0095	1.0100	1.0100	1.0110	1.0115	1.0120	1.0125	1.0130
15	1.0080	1.0085	1.0090	1.0095	1.0100	1.0105	1.0110	1.0115	1.0120	1.0125	1.0130
16	1.0085	1.0090	1.0095	1.0100	1.0105	1.0110	1.0115	1.0120	1.0125	1.0130	1.0135
17	1.0085	1.0090	1.0095	1.0105	1.0105	1.0115	1.0120	1.0125	1.0130	1.0135	1.0140
18	1.0090	1.0095	1.0100	1.0105	1.0110	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145
19	1.0090	1.0095	1.0100	1.0110	1.0115	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145
20	1.0095	1.0100	1.0105	1.0110	1.0115	1.0125	1.0130	1.0135	1.0140	1.0145	1.0150
21	1.0095	1.0100	1.0105	1.0115	1.0120	1.0125	1.0130	1.0135	1.0145	1.0150	1.0155
22	1.0100	1.0105	1.0110	1.0120	1.0125	1.0130	1.0135	1.0140	1.0150	1.0155	1.0160
23	1.0105	1.0105	1.0115	1.0120	1.0125	1.0130	1.0140	1.0145	1.0150	1.0155	1.0160
24	1.0105	1.0110	1.0120	1.0125	1.0130	1.0135	1.0145	1.0150	1.0155	1.0160	1.0165
25	1.0110	1.0115	1.0120	1.0130	1.0135	1.0140	1.0145	1.0150	1.0160	1.0165	1.0170
26	1.0115	1.0120	1.0125	1.0135	1.0140	1.0145	1.0155	1.0160	1.0165	1.0170	1.0180
27	1.0115	1.0120	1.0130	1.0135	1.0140	1.0150	1.0155	1.0160	1.0170	1.0170	1.0180
28	1.0120	1.0125	1.0130	1.0140	1.0145	1.0150	1.0160	1.0165	1.0175	1.0175	1.0185
29	1.0120	1.0130	1.0135	1.0140	1.0150	1.0155	1.0160	1.0165	1.0175	1.0180	1.0185
30	1.0125	1.0130	1.0135	1.0145	1.0155	1.0155	1.0165	1.0170	1.0175	1.0180	1.0190
31	1.0125	1.0135	1.0140	1.0145	1.0155	1.0160	1.0170	1.0175	1.0180	1.0185	1.0190
32	1.0130	1.0135	1.0140	1.0150	1.0160	1.0165	1.0170	1.0180	1.0185	1.0190	1.0195
33	1.0130	1.0140	1.0145	1.0155	1.0160	1.0165	1.0170	1.0180	1.0185	1.0190	1.0195
34	1.0135	1.0140	1.0150	1.0160	1.0165	1.0170	1.0175	1.0185	1.0190	1.0195	1.0200
35	1.0135	1.0145	1.0150	1.0160	1.0165	1.0170	1.0180	1.0190	1.0195	1.0200	1.0205
36	1.0140	1.0145	1.0155	1.0165	1.0170	1.0175	1.0185	1.0195	1.0200	1.0205	1.0210
37	1.0145	1.0150	1.0160	1.0165	1.0175	1.0180	1.0185	1.0195	1.0200	1.0205	1.0210
38	1.0150	1.0155	1.0165	1.0170	1.0175	1.0185	1.0190	1.0200	1.0210	1.0215	1.0215
39	1.0150	1.0160	1.0165	1.0170	1.0180	1.0185	1.0195	1.0205	1.0210	1.0215	1.0220
40	1.0155	1.0165	1.0170	1.0175	1.0185	1.0190	1.0200	1.0210	1.0215	1.0220	1.0230

TABLE V.—*Volume of milk and cream at various temperatures occupied by unit volume at 68° F. (20° C.)*—Continued

Percentage of butter fat.	Temperature (° F.).										
	120	122	124	126	128	130	132	134	136	138	140
	Volume.										
0.025	1.0110	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0155	1.0160	1.0170	1.0175
1	1.0110	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0155	1.0160	1.0170	1.0175
2	1.0115	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0155	1.0160	1.0170	1.0175
3	1.0115	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0155	1.0160	1.0170	1.0175
4	1.0115	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0155	1.0160	1.0170	1.0175
5	1.0115	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0155	1.0160	1.0170	1.0175
6	1.0115	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0155	1.0160	1.0170	1.0175
7	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0150	1.0155	1.0160	1.0170	1.0175
8	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0150	1.0155	1.0165	1.0170	1.0175
9	1.0120	1.0130	1.0135	1.0140	1.0145	1.0150	1.0155	1.0160	1.0165	1.0170	1.0180
10	1.0120	1.0130	1.0135	1.0140	1.0145	1.0150	1.0155	1.0160	1.0165	1.0170	1.0180
11	1.0120	1.0130	1.0135	1.0140	1.0145	1.0150	1.0155	1.0160	1.0165	1.0170	1.0180
12	1.0130	1.0135	1.0140	1.0145	1.0150	1.0155	1.0160	1.0165	1.0170	1.0175	1.0180
13	1.0130	1.0135	1.0140	1.0145	1.0150	1.0155	1.0160	1.0165	1.0170	1.0175	1.0180
14	1.0135	1.0140	1.0145	1.0150	1.0155	1.0160	1.0165	1.0170	1.0175	1.0180	1.0185
15	1.0135	1.0140	1.0145	1.0150	1.0155	1.0160	1.0165	1.0170	1.0175	1.0180	1.0185
16	1.0140	1.0145	1.0150	1.0155	1.0160	1.0165	1.0170	1.0175	1.0180	1.0185	1.0190
17	1.0145	1.0150	1.0155	1.0160	1.0165	1.0170	1.0175	1.0175	1.0180	1.0185	1.0190
18	1.0150	1.0155	1.0160	1.0165	1.0170	1.0175	1.0180	1.0185	1.0185	1.0190	1.0195
19	1.0150	1.0155	1.0160	1.0165	1.0170	1.0175	1.0180	1.0185	1.0185	1.0190	1.0195
20	1.0155	1.0160	1.0165	1.0170	1.0175	1.0180	1.0185	1.0190	1.0195	1.0200	1.0205
21	1.0160	1.0165	1.0170	1.0175	1.0180	1.0185	1.0190	1.0190	1.0195	1.0200	1.0205
22	1.0165	1.0170	1.0175	1.0180	1.0185	1.0190	1.0190	1.0195	1.0200	1.0205	1.0210
23	1.0165	1.0170	1.0175	1.0180	1.0185	1.0190	1.0195	1.0195	1.0200	1.0205	1.0210
24	1.0170	1.0180	1.0185	1.0190	1.0195	1.0200	1.0205	1.0205	1.0210	1.0215	1.0220
25	1.0175	1.0180	1.0185	1.0190	1.0195	1.0200	1.0205	1.0210	1.0215	1.0220	1.0225
26	1.0185	1.0190	1.0195	1.0200	1.0205	1.0210	1.0215	1.0220	1.0225	1.0230	1.0235
27	1.0185	1.0190	1.0195	1.0200	1.0205	1.0210	1.0215	1.0220	1.0225	1.0230	1.0235
28	1.0190	1.0200	1.0205	1.0210	1.0215	1.0220	1.0225	1.0230	1.0235	1.0240	1.0245
29	1.0195	1.0200	1.0205	1.0210	1.0215	1.0220	1.0225	1.0230	1.0235	1.0240	1.0245
30	1.0195	1.0200	1.0205	1.0210	1.0215	1.0220	1.0225	1.0235	1.0240	1.0245	1.0250
31	1.0200	1.0205	1.0210	1.0215	1.0220	1.0225	1.0230	1.0235	1.0240	1.0245	1.0250
32	1.0205	1.0210	1.0215	1.0220	1.0225	1.0230	1.0235	1.0240	1.0245	1.0250	1.0255
33	1.0205	1.0210	1.0215	1.0220	1.0225	1.0230	1.0235	1.0240	1.0245	1.0250	1.0255
34	1.0210	1.0215	1.0220	1.0225	1.0230	1.0240	1.0245	1.0245	1.0250	1.0255	1.0260
35	1.0210	1.0215	1.0220	1.0225	1.0230	1.0240	1.0245	1.0250	1.0250	1.0255	1.0260
36	1.0215	1.0225	1.0230	1.0235	1.0240	1.0245	1.0250	1.0255	1.0260	1.0265	1.0270
37	1.0215	1.0225	1.0230	1.0235	1.0240	1.0245	1.0250	1.0255	1.0260	1.0265	1.0270
38	1.0220	1.0230	1.0235	1.0240	1.0245	1.0250	1.0255	1.0260	1.0265	1.0270	1.0280
39	1.0225	1.0235	1.0240	1.0245	1.0250	1.0255	1.0260	1.0265	1.0270	1.0275	1.0280
40	1.0235	1.0240	1.0245	1.0255	1.0260	1.0265	1.0270	1.0275	1.0280	1.0285	1.0290

LIFE HISTORY OF THE MELON FLY

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INTRODUCTION

Aside from the Mediterranean fruit fly, *Ceratitis capitata* Wied., there is no other insect in the Hawaiian Islands that is causing such financial loss to fruit and vegetable interests as the melon fly, *Bactrocera cucurbitae* Coq. The damages caused by its ravages are placed by some even higher than those caused by *C. capitata*. While *B. cucurbitae* was not officially recorded until November, 1898, when it was first discovered by Mr. George Compere in the market gardens in the environs of Honolulu, it had been knownlocally about that city many years before. Mr. Albert Waterhouse, Acting President of the Hawaiian Board of Agriculture and Forestry, states that less than 30 years ago excellent cantaloupes (*Cucumis melo*) and watermelons (*Citrullus vulgaris*) and many kinds of pumpkins and squashes were grown in profusion the year round. Since that time the spread of the melon fly has been so rapid that this insect is now found on all the important islands of the Hawaiian group, and cantaloupes and watermelons can not be grown except on new land distant from old gardens. More than 95 per cent of the pumpkin (*Cucurbita pepo*) crop is annually ruined, not to mention the havoc caused among the more resistant cucumbers (*Cucumis sativus*).

Not only does the adult melon fly oviposit in fruit that has already set, but more often—in the case of the pumpkin and the squash (*Cucurbita* spp.)—in the unopened male and female flowers, in the stem of the vine, and even in the seedling itself, especially in seedlings of the watermelon and the cantaloupe. The writers have observed entire fields of watermelons killed before the plants were 6 to 8 inches long by larvæ boring into the taproot, stem, and leaf stalks. An examination of almost any pumpkin or squash field in the agricultural districts on the Island of Oahu at certain seasons of the year will show that very nearly all the flowers are affected before they have an opportunity to bloom, In about 95 cases out of 100 the anthers of the male bloom are either reduced to a mass of rot or more or less eaten before the bud becomes full grown, and the young ovaries of the female bloom are ruined by the burrowing maggots either before or shortly after the flower unfolds.

While cucurbitaceous crops are the favored host fruits of the melon fly, certain varieties of leguminous crops, such as string beans and cowpeas, are often badly attacked. When preferred host fruits are scarce, even peaches, papayas, and similar fruits are attacked to a limited degree. No satisfactory remedy has yet been found to prevent the infestation of fruit. The Chinese gardeners save a small percentage of crops subject to the attacks of this pest by covering the young fruit with cloth or paper or, in the case of the curcurbits, by burying them in the soil until they become sufficiently large to withstand attack.

The female melon fly deposits her eggs in small batches just beneath the surface of the fruit, vegetable, or plant affected. From these eggs

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maggots are hatched which feed and burrow about, causing the rapid destruction of the affected parts, and then leave the host to enter the soil, where they pupate. After a short time the adult emerges from the pupa and soon deposits eggs for the following generation of larvæ.

THE EGG

From the data included in Table I it will be seen that the duration of the egg stage of the melon fly is very short. During the warm summer months, when the daily mean temperature is about 79° F., eggs hatch in 26 to 35 hours after deposition. The data indicate that hatching proceeds most rapidly about 27 or 28 hours after the eggs are laid. At a mean temperature of 75.6°, 84 eggs hatched in from 31 to 38 hours, while at a mean of 75°, 96 eggs hatched in about 47 hours after deposition. At 73.6°, 88 eggs hatched about 52 to 54 hours after being laid.

TABLE I.—*Duration of the egg stage of the melon fly*

Number of eggs under observation.	Eggs deposited.		Eggs hatched.		Average mean temperature for period.
	Day.	Period.	Day.	Period.	
					°F.
17	Aug. 20	10.30 to 11.30 a. m.	Aug. 21	1 to 2 p. m.	79.5
75	...do	...do	...do	2 to 2.15 p. m.	79.5
45	...do	...do	...do	2.30 to 2.45 p. m.	79.5
12	...do	...do	...do	2.45 to 3 p. m.	79.5
23	...do	...do	...do	3 to 3.15 p. m.	79.5
9	...do	...do	...do	3.15 to 3.30 p. m.	79.5
6	...do	...do	...do	3.30 to 3.45 p. m.	79.5
20	...do	...do	...do	3.45 to 4 p. m.	79.5
9	...do	...do	...do	4 to 4.30 p. m.	79.5
37	...do	...do	...do	4.30 to 6 p. m.	79.5
7	...do	...do	...do	6 to 8 p. m.	79.5
5	...do	...do	...do	8 to 9 p. m.	79.5
2	...do	...do	...do	9 to 10 p. m.	79.5
84	May 19	3.15 to 3.30 p. m.	May 20	10 p. m. to 2 a. m.	75.6
96	May 14	4 to 6 p. m.	May 16	3 to 5.30 a. m.	75.0
13	May 13	10 a. m. to 1 p. m.	May 14	About 9 p. m.	75.5
62	...do	...do	...do	10 p. m. to 3 a. m.	75.5
77	May 11	11.30 a. m. to 2 p. m.	May 13	3 to 6 a. m.	73.6
11	...do	...do	...do	6 to 9.30 a. m.	73.6

THE LARVA

The larva of the melon fly passes through three instars before being full grown. The data in Table II show that at a mean temperature of about 79° F. larvæ can complete their development in from four days and four hours to seven days. The larvæ recorded as feeding upon papaya (*Carica papaya*) were transferred several times a day from one small piece of pulp to a fresh piece; hence, they probably pupated a few hours sooner than they would have pupated had they undergone their entire development in a single fruit. Larvæ developing in thick-skinned fruits, such as watermelons and pumpkins, often remain in the fruit after becoming full grown several days longer before emerging to pupate than they would have done had they been less confined. During the cooler seasons of the year the length of the larval life will probably be found to be much longer.

TABLE II.—*Duration of the larval stage of the melon fly*

Number of specimens under observation.	Approximate period of development.	Host fruit.	Number of hours in—			Period in larval stage.		Average mean temperature for period of development.
			Instar 1	Instar 2	Instar 3			
						Days.	*Hours.*	°F.
1	Sept. 25 to 29.	Papaya	26	24	50	4	7	78. 2
1	do	do	24	25	54	4	7	79
1	do	do	25	25	51	4	10	79
1	Sept. 25 to 30.	do	27	25	60	4	16	79
1	do	do	26	40	52	4	22	79
1	Sept. 29 to Oct. 4.	do	24	24	60	4	12	78. 2
1	do	do	23	23. 5	60	4	10	78. 2
1	do	do	23. 5	22	60	4	10	78. 2
1	do	do	23. 5	23. 5	60	4	11	78. 2
1	do	do	22. 5	23	60	4	11	78. 2
1	do	do	25. 1	36	60	5	1	78. 2
12	Aug. 22 to 26.	Cantaloupe				5		79
55	Aug. 22 to 27	do				6		79
28	Aug. 22 to 28.	Cucumber				6		79
42	Aug. 22 to 29.	Cantaloupe				7		79
36	do	Cucumber				7		79

THE PUPA

At mean temperatures varying from 71.6° to 79.4° F., the pupal stage ranges from 7½ to 13 days, as determined by observations on the 1,400 pupæ recorded in Table III. As the mean winter monthly temperatures seldom fall below 70° F., 13 days is probably close to the maximum length of the pupal stage during the cooler seasons in littoral Hawaii.

TABLE III.—*Duration of the pupal stage of the melon fly*[a]

Date of pupation.	Date of emergence.	Number of adults emerging.	Number of days of pupal stage.	Average mean temperature.
				°F.
Aug. 14, a. m	Aug. 22, a. m	5	8	78. 7
Do	Aug. 23, a. m	25	9	78. 9
Do	Aug. 25, a. m	1	11	78. 9
Aug. 17, a. m	do	60	8	79. 3
Do	Aug. 25, p. m	1	8. 5	79. 3
Do	Aug. 26, a. m	27	9	79. 2
Aug. 18, p. m	do	2	7. 5	79. 3
Do	Aug. 27, a. m	10	8. 5	79. 4
Sept. 17, a. m	Sept. 26, a. m	26	9	79. 2
Do	Sept. 27, a. m	37	10	79. 2
Sept. 20, a. m	Sept. 29, a. m	355	9	79. 4
Do	Sept. 30, a. m	33	10	79. 3
Sept. 19, a. m	Sept. 28, a. m	300	9	79. 4
Do	Sept. 29, a. m	15	10	79. 3
Feb. 3, a. m	Feb. 15, a. m	45	12	71. 6
Do	Feb. 16, a. m	192	13	71. 6
Feb. 4, a. m	do	38	12	71. 6
Do	Feb. 17, a. m	228	13	71. 6

[a] Total number of pupæ under observation: 1,400.

THE ADULT

The adults of the melon fly have proved most interesting from the standpoint of general hardiness, longevity, and oviposition.

LONGEVITY.—At the present time (Aug. 30, 1914) the writers have about 205 adults that emerged on February 17. They are, therefore, 6 months and 14 days old and are as strong and vigorous in appearance and action as when they emerged. Of the 248 adults alive on June 19, but 15 females and 28 males have died to date. If the death rate continues as low in the future, a few adults will probably live to be a year old.

SEXUAL MATURITY.—Neither male nor female melon flies are sexually mature when they emerge from the pupa. Out of about 200 individuals emerging on May 24, one pair was noted in coition on June 13, or 20 days after emergence. Among a second lot of adults, emerging on May 23, no adults mated until June 16, when two pairs were seen in coition. The majority of females in these lots did not mate until fully 25 days old. The daily mean temperatures for the period from May 23 to June 16 averaged 75.5° F. Sexual activity begins only at sunset. From sunset to dark copulation occurs and lasts in many instances until daybreak.

OVIPOSITION.—At mean temperatures averaging 75.5° F., females did not begin egg laying until about one month after eclosion. While fruit was placed in jars with about 1,000 adults which emerged from May 23 to 25, no attempts at oviposition were noted until June 23, when 12 punctures containing no eggs were made in a mango by females that emerged on May 24. The first eggs, 12 in number, were laid on June 25, or 32 days after eclosion. No eggs were obtained from females that emerged from May 25 until June 28, or 34 days after eclosion.

DAILY RATE OF OVIPOSITION.—While females do not begin ovipositing until about 1 month old, they continue to lay eggs for a long period. Thus, in Table IV is recorded the daily rate of oviposition of seven females during the first three months after emergence, while in Table V is recorded that of three females during the fourth, fifth, and sixth months of their life.

TABLE IV.—*Daily rate of oviposition of the melon flies that emerged on May 25 and were placed separately with fruit on June 25, 1914*

Date of observation.	Number of eggs deposited.						
	Fly No. 1.	Fly No. 2.	Fly No. 3.	Fly No. 4.	Fly No. 5.	Fly No. 6.	Fly No. 7.
July 10						23	
11			13		17		
12							
13							
14							
15	14					12	
16							
17			9		14		
18	19						
19							19
20							
21						6	
22	13						
23					10		

TABLE IV.—*Daily rate of oviposition of the melon flies that emerged on May 25 and were placed separately with fruit on June 25, 1914*—Continued

Date of observation.	Number of eggs deposited.						
	Fly No. 1.	Fly No. 2.	Fly No. 3.	Fly No. 4.	Fly No. 5.	Fly No. 6.	Fly No. 7.
July 24				3			
25							
26						23	
27	29						
28							
29					21		
30							
31							
Aug. 1				10		5	
2			15				
3							
4		2			15		
5				25			
6	16						
7		6					
8					10	8	
9	19				3		
10							
11				6		1	
12					2		
13					10		
14	16						23
15			10				
16						1	
17					5	2	9
18				11			
19	12						
20							
21							
22							
23						8	
24	17	12			9		
Total	155	20	47	55	116	89	51

TABLE V.—*Daily rate of oviposition of melon flies that emerged on February 17 and were placed separately with fruit on May 22, 1914*

Date.	Number of eggs deposited.			Date.	Number of eggs deposited.		
	Fly No. 1.	Fly No. 2.	Fly No. 3.		Fly No. 1.	Fly No. 2.	Fly No. 3.
May 22	21			June 1			
23				2			
24				3			
25				4			
26				5			
27				6		17	
28				7			
29				8		13	
30	14	2		9			
31	4		2	10			

TABLE V.—*Daily rate of oviposition of melon flies that emerged on February 17 and were placed separately with fruit on May 22, 1914*—Continued

Date.	Number of eggs deposited.			Date.	Number of eggs deposited.		
	Fly No. 1.	Fly No. 2.	Fly No. 3.		Fly No. 1.	Fly No. 2.	Fly No. 3.
June 11	.....	.....	.....	July 17	.....	.....	.....
12	.....	.....	.....	18	.....	.....	.....
13	.....	.....	.....	19	.. 16	.....	.....
14	.....	.....	.....	20	.....	.....	.....
15	.....	.....	9	21	2	.....	.....
16	1	.....	.....	22	.....	.....	.....
17	.....	12	.....	23	.....	.....	.....
18	.....	.....	.....	24	.....	14	.....
19	.....	.....	.....	25	.....	.....	.....
20	.....	.....	.....	26	.....	.....	17
21	10	.....	.....	27	8	28	13
22	.....	.....	.....	28	.....	.....	.....
23	.....	4	.....	29	.....	.....	.....
24	.....	.....	.....	30	.....	.....	4
25	.....	.....	.....	31	13	.....	.....
26	.....	.....	.....	Aug. 1	.....	.....	.....
27	.....	.....	.....	2	.....	.....	.....
28	.....	.....	10	3	13	.....	.....
29	.....	.....	.....	4	.....	.....	12
30	.. 18	.....	.....	5	.....	.....	.....
July 1	.....	.....	.....	6	.....	.....	.....
2	.....	.....	.....	7	.....	.....	.....
3	.....	.....	.....	8	.....	8	17
4	.....	.....	.....	9	8	.....	.....
5	.....	.....	.....	10	10	.....	21
6	.....	.....	.....	11	.....	.....	.....
7	.....	.....	.....	12	10	.....	21
8	.....	.....	.....	13	.....	.....	.....
9	.....	7	.....	14	.....	.....	.....
10	.....	.....	.....	15	.....	.....	22
11	.....	.....	.....	16	.....	12	.....
12	.....	.....	.....	17	12	.....	.....
13	3	.....	.....	18	.....	.....	14
14	.....	.....	.....				
15	.....	16	.....	Total	166	133	169
16	3	.....	.....				

While the individuals whose rate of oviposition is recorded in Table V were not given an opportunity to oviposit during the first three months, it will be noted that they oviposited quite as freely at the end of the sixth month as did the youngest females. There is no reason to believe that the first group of melon flies (Table IV) will not continue to oviposit, since the second group (Table V), even before they had an opportunity to oviposit in fruit, oviposited on the sides of the jar containing them. It will be noted that the female melon fly oviposits very irregularly. She is inclined to deposit a large number of eggs at greater intervals, whereas the female of the Mediterranean fruit fly deposits a smaller number nearly every day. Thirty-six eggs is the largest number ever secured from one melon fly in one day. The greatest daily average thus far recorded is that of fly No. 1 of Table IV, which laid a total of 155 eggs during the first 46 days after she commenced ovipositing, an average of 3.4 eggs per day.

IDENTIFICATION OF THE SEEDS OF SPECIES OF AGROPYRON

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INTRODUCTION

The identification of the "seeds" [2] of the species of Agropyron is an important problem to the farmer, the seedsman, and the seed laboratory. *Agropyron repens* (quack-grass) is recognized as a dangerous weed, and the similarity between the seed of this species and other common but more desirable species of Agropyron gives rise to confusion. Up to the present time no diagnosis has been discovered which appears to be entirely satisfactory for use in seed-laboratory practice. This paper deals only with the seeds of the species of Agropyron which are common in the Minnesota seed trade—viz, (1) quack-grass (*A. repens* (L.) Beauv.); (2) western wheat-grass (*A. smithii* Rydb.); and (3) slender wheat-grass (*A. tenerum* Vasey). The diagnosis here presented has proved to be not only sound but easily applied in many hundreds of tests made at the Minnesota Seed Laboratory.

HISTORICAL REVIEW

The species of Agropyron are quite easily distinguishable from each other when characteristics of the root systems, leaf characters, spikes, and spikelets are taken into consideration, and these differences are described in standard works on the taxonomy of the flowering plants (1, 2, 3, 4).[3] In so far as the seeds are concerned, however, these published descriptions are not sufficiently detailed for use as a basis for identification of seeds alone. Hillman (5) published detailed descriptions of Agropyron spikelets which agree in every way with the observations of the writer, but do not include individual seed characters in sufficient detail for an accurate diagnosis of the seeds alone.

Stevens (8, p. 113) describes in some detail the empty flowering glumes, the size and shape of the seeds, and the rachilla of several species of Agropyron. His descriptions, except as to the rachilla, agree essentially with those of the writer. The rachilla is described by Stevens as follows:

> The footstalk (rachilla) in *Agropyron repens* is entirely smooth, while in *Agropyron occidentale* [4] it is rather variable, but commonly with scattered, short, stiff hairs.

While this may appear to be true when seeds are examined under a low-power lens, yet the rachilla of seeds of *A. repens* when magnified

[1] The writer wishes to acknowledge the assistance of Dr. E. M. Freeman, Assistant Dean, and Chief of the Division of Botany and Plant Pathology, and Mr. W. L. Oswald, Chief of the Seed Laboratory, Minnesota Experiment Station, in planning the work and giving suggestions.

[2] The word "seeds" is used in this paper in its commercial sense and includes the grain, or caryopsis, inclosed in its persistent glumes, lemma, and palea, with the persistent rachilla segments.

[3] Reference is made by number to "Literature cited," p. 281.

[4] Syn. *Agropyron smithii*.

to about 32 diameters exhibits very definite hairy characters, as described below.

Pammel and King (6, p. 170) published a brief account of the seeds of *A. repens* and *A. smithii* in which the main points of difference are pointed out as being found in the shape of the palea and in the hairs on its face and edge. While these distinctions are in the main correct, they are insufficient for a complete diagnosis. As to shape of the seeds, these authors make the following statement:

> The seed of quack grass is more slender and spindle-shaped, while that of western wheat grass broadens out somewhat toward the tip, after the manner of brome and some other grasses.

According to the results of the examinations of a large number of seeds, the writer finds that the difference in shape above noted is not constant. Hence, its use as a single determining character is not warranted.

In a taxonomic key of seeds of Agropyron issued by Sarvis (7, p. 2) the rachilla of *A. repens* is described as being "puberulent, each hair being glandular at the base." According to the writer's observations, the rachilla would more properly be described as hirsutulous. Moreover, in order to discern the glands at the base of the hairs, a compound microscope is required, which makes the use of this character impracticable for ordinary seed-laboratory methods. Even with such high power, the glands are not always clearly discernible. A glabrous palea in *A. repens* and a hispid palea in *A. smithii* are indicated by Sarvis as important characters in the determination of the Agropyron species. While this is true for the majority of seeds of these species, the writer has found many seeds in which this distinction does not hold. These characters intergrade to such an extent that they are not only unreliable, but are misleading as a single diagnostic criterion. Sarvis also holds that the tip of the palea of *A. tenerum* is very puberulent. This is true not only for the species above mentioned but also for *A. repens* and *A. smithii*.

SEED CHARACTERS OF SPECIES OF AGROPYRON

In the determination of seeds of Agropyron there are no absolutely fast and definite single characters by which a seed of one species may be unfailingly distinguished from the seed of any other species. Variation is found not only in the seed but also in the other unit parts of the plants, particularly in the spikes and spikelets (Pls. XXXIV, XXXV, and XXXVI). Moreover, seeds growing in different localities may exhibit considerable variation. This variation necessitates a close study of numerous characters of each seed, and any diagnosis to be of value must be based on a large number of seeds collected from a wide range and under widely differing conditions.

It is also obvious that the larger the number of seed characters which are studied the greater will be the possibility of making an accurate determination of the species under examination. A single character may vary to such an extent as to be quite untypical of the species, and consequently a determination based upon only one character may be incorrect, and therefore misleading. The necessity for an intimate knowledge of several distinguishing characters is even more

pronounced when Agropyron seeds become so mutilated that portions of the glumes are destroyed, as is frequently the case in commercial seed mixtures. In some cases the glumes are entirely gone, leaving only the grain, and determinations according to characters described below then become impossible. No satisfactory method of real practical value has yet been worked out whereby the seeds without the glumes may be accurately determined, and it seems probable that in such cases one may be compelled to resort to microscopic sections. One characteristic difference may be noted, however—namely, that the color of the matured grain of *A. tenerum* is somewhat lighter than that of either *A. repens* or *A. smithii*. The two latter approximate each other closely in color.

SOURCE OF SEEDS

Materials for this work were secured from as many sources as possible, as given in Table I. Only those samples have been considered which were obtained from and determined by a competent botanist, who was sure of the origin of the seed.

TABLE I.—*Sources of seeds of Agropyron spp. used in investigation*

Source of seed.	A. repens.	A. smithii.	A. tenerum.
Canada (Manitoba)	.....	.....	(a)
Illinois (Chicago) [b]	.....	(a)	(a)
Iowa	(a)	(a)	(a)
Michigan	(a)	.....	.....
Minnesota	(a)	(a)	(a)
New York (Geneva)	(a)	.....	(a)
North Dakota	(a)	(a)	(a)
North Dakota [b]	(a)	.....	(a)
Russia [b]	(a)	.....	.....
Washington (State)	(a)	(a)	(a)
Wisconsin [b]	.....	(a)	(a)
Wisconsin	(a)	.....	(a)
Wyoming	.....	(a)	(a)

[a] Sample received.
[b] From Seed Laboratory of United States Department of Agriculture, Washington, D. C.

LABORATORY METHODS OF IDENTIFICATION

It is obviously necessary that all methods of identification, especially for use by farmers or seedsmen and even for seed laboratories, be as simple as possible and that they do not require elaborate or expensive apparatus. If, however, the distinguishing characters are not visible to the naked eye or with the aid of an ordinary magnifying glass, then it becomes absolutely necessary to use a higher power of magnification.

In the identification of seeds of *Agropyron* spp. it is advisable to use a magnification of about 32 diameters for the best results. The Greenough binocular giving the stereoscopic view has proved very satisfactory. It is absolutely necessary when examining seeds under the lens to place them so that the base of the seed is toward the light, in which position the light will be properly reflected from the hairs, making them appear clear and well defined.

SHAPE OF SEED

The seed of *A. repens* (fig. 1, *A*) has its point of greatest divergence about midway between the base and the tip, differing in this respect from *A. tenerum* (fig. 1, *C*), which has its point of greatest divergence about one-third of the length of the seed from the tip. The lemma and the palea of the latter species flare out more or less at this point, thus making the seed look flattened and thin. In the majority of cases the seed is unsymmetrical in shape, the top portion of the glumes being affected by a lateral displacement, as shown in the illustration. This makes possible a quick and accurate determination of a bulk lot of seeds of *A. tenerum*. The seed of *A. smithii* (fig. 1, *B*) has the same general shape as that of *A. repens*, but it is larger and has a more robust appearance.

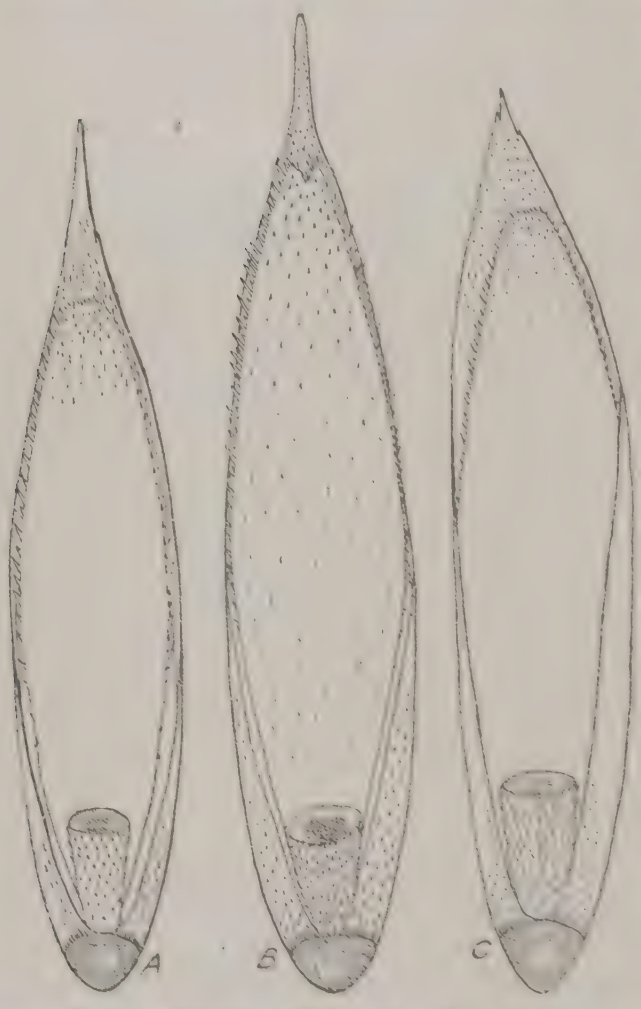

FIG. 1.—Detail drawings of dorsal view of *Agropyron* spp. *A*, *Agropyron repens*; *B*, *A. smithii*; *C*, *A. tenerum*. × 6.

RACHILLA

It is impossible to describe very definitely the characteristics of the rachilla of the different species of Agropyron because of the variation. In a general way the sides of the rachilla of *A. repens* are more nearly parallel, and the rachilla itself is more or less appressed to the palea. In *A. smithii* the sides of the rachilla diverge noticeably more from the point of its attachment. The rachilla stands out more prominently from the seed, being materially different in this respect from *A. repens* (fig. 2). The rachilla of *A. tenerum* has no particularly characteristic shape, varying from the slender to the short, stout, diverging type. A very good idea of relative size and shape of these seeds may also be gained by studying Plate XXXVII, which shows typical seeds, together with a typical spikelet of each of the three species.

The hairs clothing the rachilla constitute a valuable character used in the determination of the seed. However, care and good judgment must be exercised because of the great variation which may occur.

The characteristic rachilla of *A. repens* (fig. 1, *A*) is sparsely covered with short, minute hairs having a rather large base. Occasionally a glandular structure may be discerned at the base. This, however, can only be seen with a high-power lens and is not considered of sufficient

importance to warrant its use as a determining character. No rachilla of *A. repens* has been found which had the hirsute character of *A. smithii* (fig. 1, *B*) or the pilose character of *A. tenerum* (fig. 1, *C*).

The rachilla of *A. smithii* is characterized by hairs of the same general shape as the hairs found on the rachilla of *A. repens*. They are, however, larger and stronger and the number is noticeably greater. This characteristic is fairly uniform.

The rachilla of *A. tenerum* is characterized by hairs of a pilose nature. They are long as compared with those of *A. repens* and *A. smithii*, and may often be distinguished by this feature alone from these two species, as the pilose nature has never been observed on them. However, an absolutely authentic specimen of *A. tenerum* has been examined which had a rachilla much resembling that of *A. repens*. The hairs were short, but were not as large as the base. Other characters on the seed, however, made it possible to place it accurately in the species *tenerum*.

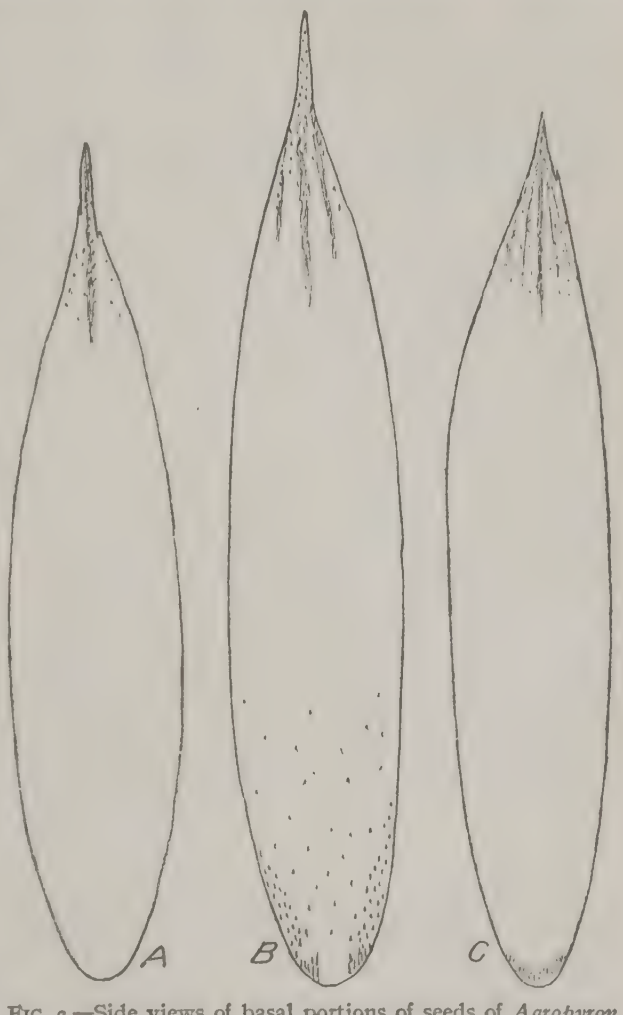

FIG. 2.—Side views of basal portions of seeds of *Agropyron* spp., showing the relative projection of the rachilla: *A*, *Agropyron repens*; *B*, *A. smithii*; *C*, *A. tenerum*. × 9.

LEMMA

Another distinguishing character and one which is reliable as to uniformity may be found at the base of the lemma on the ventral side of the seed. In *A. tenerum* (figs. 2, *C*, and 3, *C*) there is a line of hairs which extends from the base of the rachilla on the dorsal side of the seed around and entirely across the face of the lemma on the ventral side near the base of the seed. In some cases it may be impossible to distinguish the hairs on the middle of the lemma, but the surface of the lemma at this point is roughened sufficiently so that it is noticeable. This is a fairly definite character.

The seed of *A. repens* (figs. 2, *A*, and 3, *A*) has no such characteristic line of hairs, but the basal portion of the lemma is entirely smooth and shiny. This character in the seed of *A. smithii* (fig. 3, *B*) is somewhat variable and is therefore not of much value. Most commonly, however, it is found that the ring of hairs extends part way around on either side, and on the middle of the lemma there is a space which usually is entirely smooth.

PALEA

The part of the seed which discloses good and reasonably definite characteristic differences is the palea. The face of the palea in *A. repens* and *A. tenerum* (figs. 1, *A*, and 1, *C*) is practically glabrous, except near the tip, where it is puberulent. Occasionally there is a small number of hairs distributed over the face of the palea. Since the tips of the paleæ in both of these species are always puberulent, this can not be used as a distinguishing character. The palea of the seed of *A. smithii* (fig. 1, *B*) is quite hirsute over its entire surface.

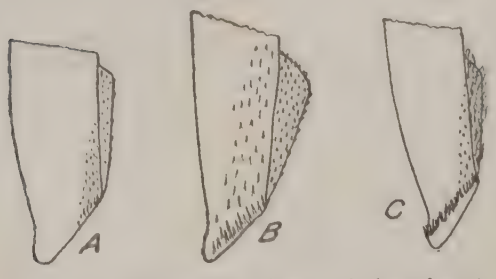

FIG. 3.—Detail drawings of ventral view of seeds of *Agropyron* spp.: *A*, *Agropyron repens*; *B*, *A. smithii*; *C*, *A. tenerum*. × 9.

The hairs on the edge of the palea have a distinctive shape for each of the three species and are very useful as a determining factor. Those of *A. repens* (fig. 4, *A*) are rather short, stout, and somewhat blunt. Those of *A. smithii* (fig. 4, *B*) are about as coarse as those on *A. repens*, but are noticeably longer, thus making them appear more slender. On *A. tenerum* (fig. 4, *C*) the hairs are finer, closer together, and more acutely pointed than in the case of the two others.

The palea of *A. smithii* (fig. 1, *B*) has a very characteristic tip, which character runs fairly uniform throughout the species. The tip of the palea is definitely divided, making a well-defined cleft (Pl. XXXVII, 2, *a*). In some cases it is rather difficult to distinguish this cleft, as the lobes may be slightly overlapped. The tips of the palea of *A. repens* and *A. tenerum* are simply rounded or only slightly indented.

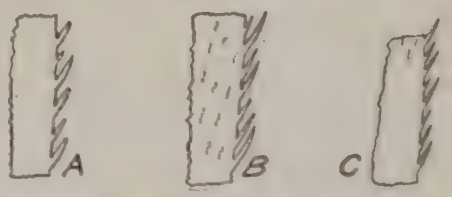

FIG. 4.—Edge of rachilla in *Agropyron* spp., showing shape and comparative size of bristles; *A*, *Agropyron repens*; *B*, *A. smithii*; *C*, *A. tenerum*. × 9.

SUMMARY

It is possible by careful examination to distinguish in commercial seed mixtures the seeds of the three species of Agropyron: *A. repens*, *A. smithii*, and *A. tenerum*.

There is no one character which can unfailingly be relied upon for this diagnosis, but the combined characters of lemma, palea, and rachilla are necessary for a safe determination.

Probably the nearest approach to a single critical structure is found in the palea, which exhibits fairly definite characters in each of the species.

The diagnostic differences are summarized in Table II.

TABLE II.—*Diagnostic differences of Agropyron spp.*

Character.	A. repens.	A. smithii.	A. tenerum.
Shape of seed	Boat-shaped	Boat-shaped	Widest one-third of distance from the tip, which is more or less flattened.
Rachilla	Sides approximately parallel. Hairs few, short, and stout.	Sides divergent. Hairs numerous, stout, but longer than those which characterize A. repens.	Variable in shape and size. Hairs numerous, slender, and long.
Palea:			
Face	Puberulent at tip; otherwise glabrous.	Hirsute over entire face.	Puberulent at tip. Remaining surface glabrous.
Edges	Characterized by short, stout, and blunt hairs.	Hairs stout, but longer than those of A. repens.	Hairs fine, acute, and close together.
Tip	Rounded or indented	Cleft	Rounded or indented.
Lemma	Smooth and shiny at base on ventral side.	Usually with a break in the line of hairs on ventral side at base of seed.	Line of hairs extends across lemma at its base.

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PLATE XXXIV

Agropyron repens: Spikes showing degrees of variation which may occur. *A*, typical spike. A side view of this spikelet may be seen at top portion of this spike. Natural size.

A

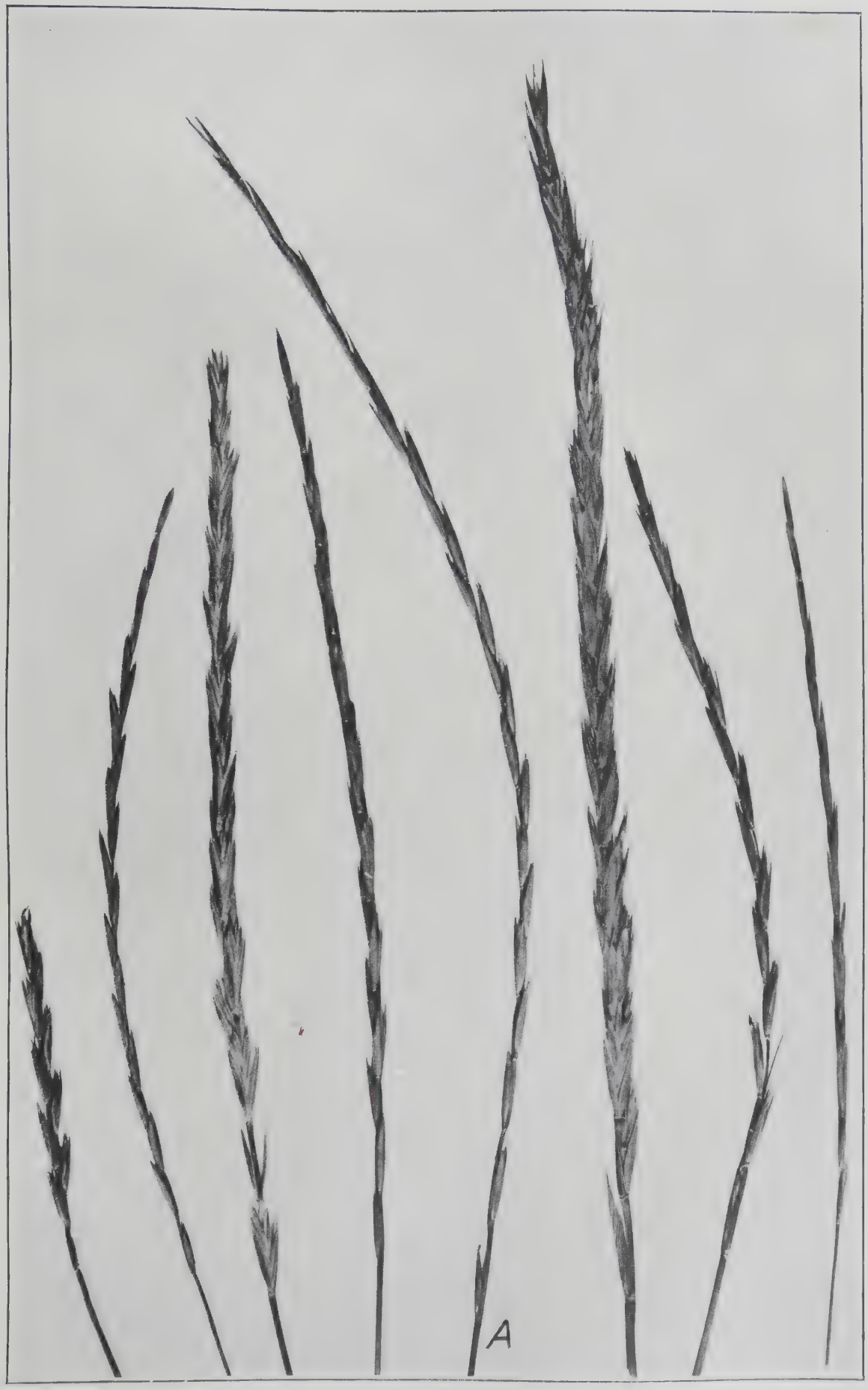
A

PLATE XXXV

Agropyron smithii: Spikes showing degrees of variation. A_1 typical spike. Natural size.

PLATE XXXVI

Agropyron tenerum: Spikes showing degrees of variation. A, typical spike. Natural size.


A

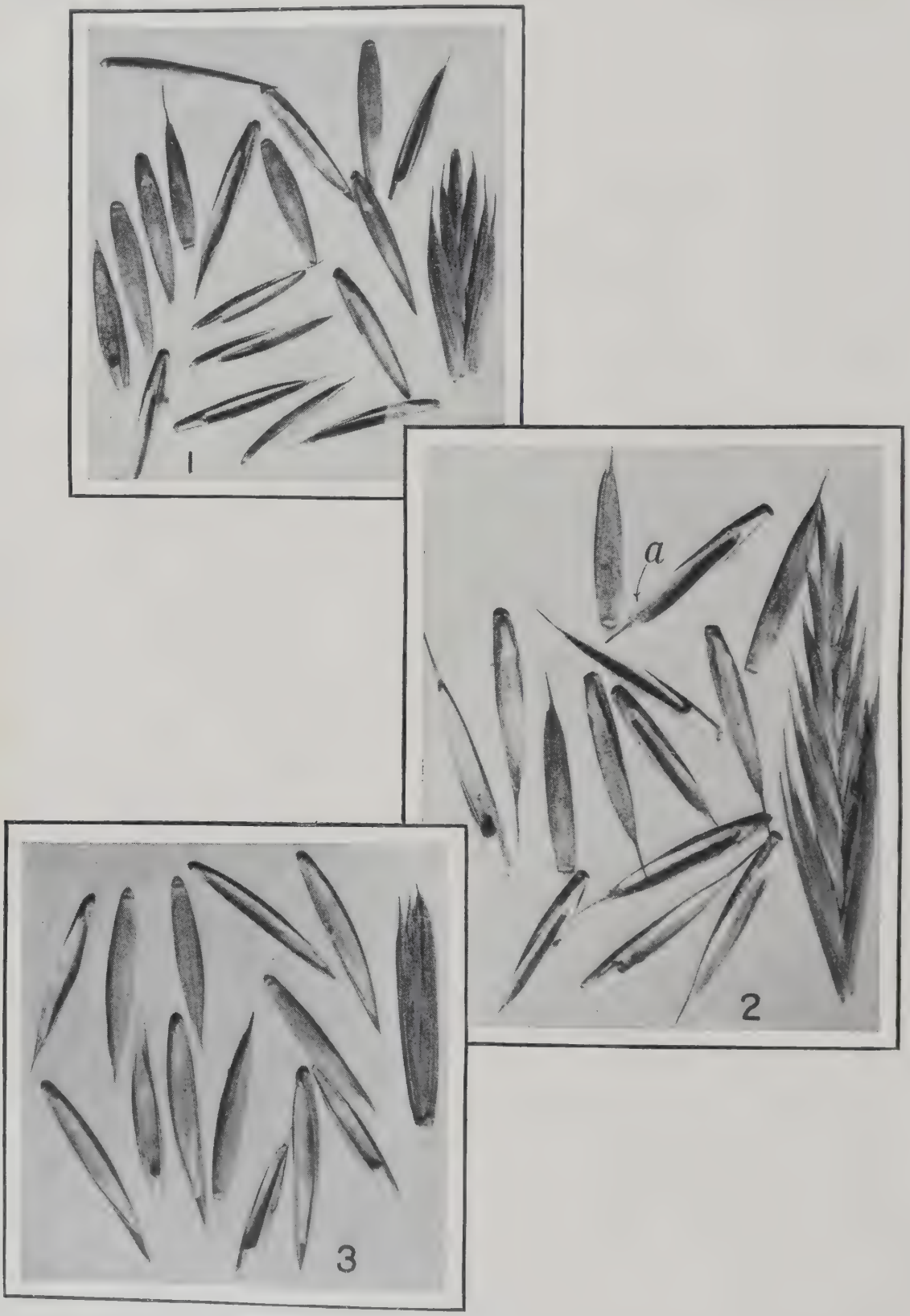

1
a
2
3

PLATE XXXVII

Agropyron spp.: Typical seeds and spikelets. Enlarged.

Fig. 1.—*Agropyron repens*.
Fig. 2.—*Agropyron smithii*.
Fig. 3.—*Agropyron tenerum*.

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OBSERVATIONS ON THE LIFE HISTORY OF AGRILUS BILINEATUS

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INTRODUCTION

At the present time the two-lined chestnut borer, *Agrilus bilineatus* Weber, is commonly associated with the death of many oaks (*Quercus* spp.) in the southeastern part of Minnesota. In 1885 reports called attention to the damage done by this insect on oaks in Massachusetts, and since that time they have been frequently mentioned as enemies of the chestnut (*Castanea dentata*) and oak. It was not until 1897 that F. H. Chittenden [1] described the adult, the larva, and the pupa, in connection with a brief summary of its life history, so far as it was known at that time. In this article it was stated that the adults appeared in the District of Columbia from May to the middle of June and laid their eggs on trees and that the larvæ worked under the bark across the grain of the wood, making a burrow from 6 to 10 inches in length, and by the next spring had constructed a chamber in the bark of living trees, where the pupal stage of about two weeks was passed. Although the name of this beetle has been common in current entomological literature, nothing of note has been added to the knowledge of its life history since Chittenden's article.

During recent years in the neighborhood of St. Paul and Minneapolis great numbers of oaks, many of them on valuable residence property, have been killed, and their death has commonly been attributed to this pest. In some of the outlying country districts areas of several acres in extent have been completely devastated, leaving the land treeless.

The present work was begun at the University of Minnesota during the fall of 1913 at the suggestion of Prof. O. W. Oestlund, of the Department of Animal Biology, under whose direction the problem was outlined and the work on the larval and pupal stages begun. In the spring of 1914 the work was continued at the Minnesota Agricultural Experi-

[1] Chittenden, F. H. Insect injury to chestnut and pine trees in Virginia and neighboring States. *In* U. S. Dept. Agr., Div. Entom. Bul. 7, n. s., p. 67–71. 1897.

ment Station under the direction of Prof. A. G. Ruggles, in charge of the Section of Spraying and Tree Insects, Division of Entomology.

The aim of this paper is to report new observations on the life history and ecologic relations of *Agrilus bilineatus*. The descriptions of the adults, larvæ, and pupæ, already referred to, are so well known to entomologists that they will not be repeated. The drawings reproduced in Plate XXXVIII of the beetles, pupæ, larvæ, and eggs were made at the Department of Animal Biology under the direction of the author. The photographs reproduced in Plate XXXIX were made at University Farm by the Experiment Station photographer.

The egg-laying habits have been described in this article in some detail because they have not been known to literature, and the same is true of the leaf-eating habits of the adults. The same may be said of the eggs and newly hatched larvæ. Some of the observations of the life history of the larvæ have not agreed with previous descriptions, and these, together with a few additional notes on habits, are included, while others, together with the details of the pupal stage, will be deferred to a later report.

ECOLOGY

The four common species of oak in the southeastern section of Minnesota, *Quercus alba* L., *Q. macrocarpa* Michx., *Q. rubra* L., and *Q. coccinea* Wang, are subject to infestation with *Agrilus bilineatus*. It seems that the members of the black-oak group are slightly more susceptible to attack than those of the white-oak group, but in localities where the infestation is severe none of the species is exempt.

In some cases the adult borers appear to prefer trees of a certain locality, or, in other cases, certain individual trees, to others even in the immediate vicinity. In general, this preference is associated with a weakened condition of the trees, but this is by no means universal. The environmental conditions of certain localities, such as drought, crowded pasturage, or cultivation, have made nearly all the trees subject to infestation by the two-lined chestnut borer, possibly because of a general weakened condition. In other cases individual trees weakened by injury or disease have been attacked, while in still other cases trees which show no signs of weakened vitality are attacked and killed by this insect.

It has often been found that the shoestring fungus, *Armillaria mellea* Vahl, has apparently been the cause of the weakened condition of the trees, and the chestnut or oak borers have followed it. In fact, it has sometimes appeared that *A. mellea* was the primary cause of the death of the trees and that the Agrilus beetle was of only secondary importance. An example of this was found in the neighborhood of Lake Elmo, Minn., where a few dead trees were found with the fungus Armillaria, but with no traces of larvæ of the two-lined chestnut borer. These observations, together with others which showed the shoestring fungus on practically every tree on which the Agrilus beetles were ovipositing, made

it seem probable that the fungus was the primary factor in causing the death of the trees.

In the vicinity of Robbinsdale, Minn., and in other localities, the Armillaria fungus is present, but is not so apparent, and all the dead trees show traces of oak borers. Furthermore, many trees with dead trunks started up from the roots the following year and gave every evidence of suffering from the girdling of the cambium layer rather than from a root infestation with the fungus.

At Robbinsdale a tree was observed on July 27 with its leaves withering as if it had been scorched, a characteristic of trees infested with *Agrilus bilineatus*. Three days before (July 24) this tree had appeared perfectly normal, but on the 27th the entire trunk of the tree was being girdled by the beetles. It was grubbed and its roots were examined by Mr. F. J. Piemeisel, of the Division of Plant Pathology, who stated that the fungus *Armillaria mellea* was not present and could have nothing to do with the death of the tree and that the root system seemed perfectly healthy. Other trees examined since gave similar evidence.

It has not yet been possible to show any relation between the amount of fungus present on a tree and the severity of the attack by the beetles. On land that was being cleared for cultivation near Lake Elmo 40 apparently healthy trees were attacked by *Armillaria mellea*, and none were found to be free from it. Furthermore, W. H. Long states[1] that a large percentage of the oaks examined by him in the eastern section of the United States have been found to be infested with the fungus. If this is true also in Minnesota and the presence of the fungus is taken as evidence of the low vitality of the tree, all but a small percentage of the oaks in Minnesota are now susceptible to attack by *Agrilus bilineatus*, and in all these cases the beetles must be looked upon as of only secondary importance. This, however, would possibly place undue weight on the mere presence of *Armillaria mellea*.

As mentioned above, in a few cases in the vicinity of Lake Elmo the death of the trees may be due to *Armillaria mellea* alone. In the majority of cases the fungus was present, but the trees were girdled by the two-lined chestnut borer, seemingly a hastening factor, at least, in the death of the trees. There still remains the possibility that the fungus was the primary factor. There are cases, as already mentioned, where the borers alone have attacked and killed apparently normal trees when the fungus was not present. The economic importance of this condition can hardly be overemphasized, for it means that the Agrilus beetles, in spite of their supposed preference for unhealthy trees, chose one healthy tree when many trees infested with the fungus were available, indicating that the interrelation between the *Armillaria mellea* and the *Agrilus bilineatus* may not be of such primary importance as would appear at first.

[1] Long, W. H. The death of chestnuts and oaks due to Armillaria mellea. U. S. Dept. Agr. Bul. 89, p. 4. 1914.

THE ADULTS

On the 17th of June the first observations of the adult *Agrilus bilineatus* were made in the vicinity of Lake Elmo, Minn. Early in the afternoon two or three could sometimes be seen at one time on a dying tree. On the same day an adult was taken from its pupal cell in the bark, and a larva was found preparing to pupate. Since no adults were seen two days earlier while the author was collecting in the same locality, it seems that these observations fix the appearance of the adults quite definitely for this section in a normal year.

The adult borers increased in numbers until they reached their greatest abundance about the 1st of July, when as many as 10 were seen at one time on a small area of bark. In the afternoon of June 26, 80 specimens were collected near Savage, Minn. There was a noticeable decline in numbers after the first week in July, and by July 20 the last record of the adults was made. Continued careful search in the field after that time was not rewarded.

During the last days of the adults' flight many instances of apparent feebleness became evident. On one occasion, while watching Agrilus beetles, a female was seen to fly slowly toward a tree apparently intending to alight on it as usual, but the insect fell to the ground as if unable to cling to the bark. The borer then made a second attempt, which was also unsuccessful, and it was picked up from the ground where it was lying apparently exhausted. The behavior of other chestnut borers in the field and in the insectary leads to the belief that the last chapter of their history is marked by the feebleness of old age.

Agrilus adults lived about 12 days in the insectary, where they were kept in a cage inclosing an oak tree. The conditions were so favorable in the insectary that many details of the various habits could be studied to great advantage, and continuous observations throughout the season were made possible in spite of weather conditions.

The habits of the Agrilus beetles were governed with such regularity that a seemingly definite program was discovered which served as a guide for later observations. At no time during the season were beetles found, even on the sunny side of the trunks, until nearly noon. On July 7 in a place near Savage, Minn., where the adult borers were very abundant, a careful search was begun shortly after 9 o'clock in the morning and but one specimen was found at 9.30 a. m., a few at about 10 o'clock, and not until 11 o'clock were they found in their usual numbers. From this time until shortly after noon they increased in numbers; then they gradually disappeared until few were to be seen late in the day. The latest field observation was made about 6 o'clock. In the insectary the beetles were inactive in the bottom of the cage until about 8 a. m., when they would begin to fly about and feed on the foliage of the tree. Many were also observed courting during the early forenoon. The absence of the beetles from the tree trunks, which they frequent later in

the day, and their feeding habits as observed in the insectary, indicate the probability that the early part of the day is ordinarily spent in this way.

It was definitely proved that the adults of *Agrilus bilineatus* feed on the foliage. They usually eat around the margins of leaves, but also tear off the epidermis and sometimes eat nearly the entire leaf, including the midrib. Plate XXXIX, figure 1, shows a leaf on which four beetles had fed for 24 hours. They even ate the edge of a paper bag which was on the floor of the insectary, where most of the observations were made.

The difficulty of observing the feeding in the field was increased by the protective habits of the beetles. The flight to and from leaves or logs and tree trunks was ordinarily quite direct, but in some cases they hovered before lighting. When disturbed, however, they flew rapidly in a zigzag manner, which is probably of protective importance, as under such conditions it is almost impossible to follow them even with the eye. If they are startled while at rest, they fold their appendages and drop to the ground, feigning death. They dodge from side to side very quickly and run rapidly over any kind of surface. On one occasion a beetle was watched walking about inside a glass tube. It experienced no difficulty unless it tried to walk upside down upon the slippery concave surface, when it began to lose its footing. This was evidently not a new occurrence, for it immediately put one of its front tarsi to its mouth and apparently moistened it; it then reached this appendage back to rub it against the posterior ones. When all the tarsi had been treated in this way, the beetle ran about until it began to slip again, when the process was repeated.

On sunny days during the entire season males and females were courting and mating. These performances were often noticed on logs or woodpiles and on the foliage of small plants at the base of trees, as well as on tree trunks. The courting was usually abbreviated and sometimes wanting. Males were seen to fly from the air directly to females on the tree. At other times a male was seen to side-step to within an inch of a female and then spread its wings as if to fly before advancing farther.

In one case a male stood near a female until she finished ovipositing and then mated with her. But it was not always the males that made the advances, for it was not uncommon to see females courting males, in which case they often found themselves ignored. In mating, the sexes were together from 2 to 12 minutes, with an average of about 4 minutes.

The females were ovipositing from June 19 to July 13. No record of ovipositing was obtained before 11 a. m. and but one after 5.30 p. m. While it was not so common to see females actually ovipositing, they could be seen nearly all the time during the hours of activity with their ovipositors out searching for places to oviposit. They laid the eggs in the bottoms of cracks, but not every crack was suitable. The females went carefully along, using their ovipositors as tactile organs, exploring every crevice. The insect often appeared to be in a great hurry and

rushed from one crack to another until the proper place was found. During the first week of July one female took 21 minutes from the time she lighted on a tree to find a place to lay her eggs. Not a minute of this time was wasted, and many cracks were rejected before the favorable one was located.

The cracks chosen for oviposition were usually ones that were quite deep. In one case a female used a crack which had evidently been made by lightning, but in all other cases a crevice at the bottom of a deep crack between ridges of the bark was chosen (Pl. XXXVIII, fig. 1). Since the bark is usually rougher on the trunk and larger limbs, especially near the ground, more favorable places to oviposit are found on these parts of the tree. Observations were made 25 feet from the ground, but few beetles were seen and none were looking for places to oviposit. In examining trees which had been killed, it was found in one case that an egg had been laid in a crevice at the axis of a small branch 41 feet 6 inches from the ground. On one tree which was very badly infested practically every branch more than 1½ inches in diameter had burrows on it. Many attempts were made to find whether the sunny side of trees was preferred to the shady side, but, so far as could be determined, the beetles showed no preference. From this evidence it seems that the eggs may be laid on any part of the tree which affords suitable cracks and that since such cracks are most numerous on the trunk, this is the usual place for ovipositing.

The females settled down when a favorable crack was found and were apparently motionless during oviposition. The beetles stood in any convenient position during the process, and there was no relation between the number of eggs laid and the length of time apparently spent in depositing them. Oviposition lasted from 1 to 5 minutes, and from 1 to 10 eggs were laid. It is probable that the number of eggs in a cluster depends upon the favorableness of the crevices in which they are deposited, because the females usually hasten to find another place as soon as one cluster has been laid. Just how many eggs are laid in all by one individual is not known.

THE EGG

Plate XXXVIII, figure 2, shows a very typical cluster of four eggs, the average number, on a bit of bark taken from the bottom of a crack, just as they appeared within an hour after they were deposited. One of the eggs lies entirely exposed, showing the typical form of an undisturbed egg, while the others illustrate how nicely they mass together and fit the irregularities of the crevices. It will be noticed that the eggs are not plump but have an unfilled, wrinkled appearance, which makes it possible for them to fit into crevices of all shapes. A typical egg, such as the exposed one in the illustration, is somewhat oval and measures about 940μ in length, 480μ in width, and about 300μ in thickness. The newly laid eggs were covered with a glistening substance which stuck them firmly in place when dry.

The eggs were hatched in the laboratory in from 10 to 13 days. It was found that the outer membrane became dry and shriveled and even cracked in from 3 to 6 days, while the inner membrane became brown and the embryo seemed to develop at one side of the egg, which became plumper than the other side (Pl. XXXVIII, fig. 3).

THE LARVÆ

When the larvæ hatched, they broke through the egg membrane on the side toward the bark and immediately began to burrow. As a result, they were not exposed to view, and the eggshells were found filled with the frass which had been burrowed out at the start. In a few cases the eggs had been entirely loosened from the bark for examination, and none of the larvæ from these eggs succeeded in getting a burrow started, except in one case where the egg was artificially fastened to the bark before the larva left it. Since the eggs are stuck to the bark in the depths of cracks, they offer a certain resistance to the larva's efforts, which makes it possible for its mandibles to get hold of the bark and start the burrow.

The newly hatched larvæ (Pl. XXXVIII, fig. 4) measure only from 1 to 1½ mm. in length, but one was found capable of reaching the cambium layer in 24 hours by burrowing for 2¼ mm. The fact that the eggs were laid in the depths of cracks made it possible for the larvæ to reach the soft cambium layer by penetrating but a few millimeters of hard bark tissue. Having reached the cambium layer they started off in any convenient direction. Observations show that burrows made during the first instar often go obliquely across the grain of the wood or with the grain, the larvæ being indifferent as to whether they go up or down the tree. If care is taken in removing the bark when green, the tiny burrows can be traced to a widening of the burrow which marks the end of the first instar. The burrows measured showed that the larvæ had burrowed for a distance of 60 to 135 mm. when the first molt took place.

In most cases the widenings in the burrow occurred when the larvæ had gone into the wood, less often into the bark, and were again returning to the cambium layer. It is evident that these points marked the limits of instars and that the molting took place in these excavations in the wood, which were often two or three times the length of the larvæ. Places were found, however, where the burrows showed that the molt had been made in the cambium layer. The longest larva found in a burrow of the first instar measured 4.6 mm. in length, and the average width of the burrows was 270μ.

The burrows made during the second instar measured about 900μ in width and took about the same course through the cambium layer, but they were about twice as long. At the beginning of the third instar quite a different course was usually found, especially in green bark on the trunks of trees, where the burrows were almost always transverse to the grain of the wood. The burrows of the fourth instar were about 2 mm. in width and often attained the length of 500 or 600 mm. Where the

bark was thick these burrows were quite generally transverse to the grain of the wood. This condition, as well as the oblique course of some of the smaller burrows, is well shown in Plate XXXIX, figure 3.

At the close of the fourth instar the larva burrows out into the bark, if it is thick enough, and constructs a cell in which it hibernates. Here pupation takes place in the spring. These cells are found in the ridges of the bark on the trunk and larger limbs of the tree and in the wood on small, thin-barked trees and limbs. In constructing the cell, the larva burrows out to within a few millimeters of the surface of the bark, withdraws itself 2 or 3 mm., then turns about to one side and excavates around the posterior portion of its body until an oblong cell has been constructed. The portion of the burrow leading to the cell, as well as the short portion between the cell and the bark, is plugged with the frass loosened in making the excavation. It is evident that at least the portion of the frass at the outer end of the cell was never ingested, for if this were the case it would have passed out at the anus, which has been at the other end of the cell all the time. When the cell is complete, it measures about 10 mm. in length and about 2 or 3 mm. in width and contains the larva bent upon itself, ready for hibernation (Pl. XXXVIII, fig. 6).

The work of the larval life is well illustrated in Plate XXXIX, figure 4, which is a reproduction of a photograph of a limb in which the entire burrow is traced, from the shells of the eggs in a crevice of the bark to the larva in its pupal cell. The burrow was carefully marked with india ink, so that the black lines represent the exact width of the burrow in every case, and at the places where the larva entered the wood to molt the holes have been marked about with white ink for the sake of contrast. The larva in this case burrowed into the wood for a short distance at first, then returned to the cambium layer, where it burrowed about until it reentered the wood to molt. From the point where the larva entered the bark to the place it emerged from the wood after the first molt the burrow measures 69 mm. in length and 270μ in width.

It will be noticed that in each succeeding instar the burrow is much wider and longer, so that in the second instar the length is 103 mm. and the width is 900μ; in the third instar it is 210 mm. long and 1.21 to 1.56 mm. in width; and in the fourth and last instar the length is 456 mm. and the width is 1.96 to 2.15 mm. The total length of this particular burrow is 835 mm., or nearly 3 feet. Since the burrows made during the early instars are so small that they are hard to find, it seems likely that Chittenden's statement [1] that the complete burrow is only 6 to 10 inches in length was based on observations made on burrows which represented the last instar. Even these could hardly have been complete, for burrows of this instar nearly 2 feet long have been found. His statement that they are for the most part transverse to the grain of the wood makes it seem even more evident that those of the last instar were described, for, as Plate

[1] Chittenden, F. H. Loc. cit.

XXXIX, figure 3, shows, the transverse direction is the usual one in the last instar, especially when the bark is thick, while during the earlier instars the burrows run in a more oblique direction.

No evidence can be offered as to the duration of instars. Larvæ which were in the first stage were found from July 21 to August 13, mature larvæ were found in their pupal cells as early as August 7, while the intermediate stages were found throughout this period.

It was found that the larvæ in the last instar burrow from 2 or 3 to 23 mm. in 24 hours. Larvæ of the second instar burrow as far as 6 mm. in the same length of time. Upon consideration of these records it is not surprising that trees infested with *Agrilus bilineatus* appear to die suddenly when larvæ are to be found as numerous as shown by the burrows in Plate XXXIX, figure 3, where each one may consume cambium tissue equal to nearly twice its own bulk every 24 hours. In the section shown there were nine larvæ in approximately 1 square foot of bark, each burrowing across the cambium layer. It was also found that when the larvæ are so numerous that they confront each other, one or the other is eaten through as if it were merely cambium tissue. This may become an important economic factor, for cases have been observed in bark which was crowded with grubs where a number of dead larvæ were found with burrows passing through their abdomens or even their heads.

The slowest burrowing was found to be in the dry wood, where the tissue was evidently the toughest and of the least nutritive value. Trees with growing tissue offer the best opportunity for making extended burrows with great nutritive value to the larvæ and to the detriment of the tree. On the other hand, as soon as the tree dies from being girdled, the tissue becomes dry and offers more resistance to the burrowing and is of little nutritive value to the larvæ, which may die. A tree which was grubbed up on July 30, at which time it had just died, was examined on August 13. The dried bark, especially on the side exposed to the sun, contained shriveled larvæ, over 50 per cent of which were dead. Similar conditions were found in other trees that had died early in the season, when the dryness seemed to affect the larvæ more than later when they are in the pupal cells. This may also explain the condition described by Chittenden, who stated that burrows were found in trees, but no larvæ were present.[1]

In summarizing the work of the larvæ of the two-lined chestnut borer it is also of economic interest to note the wide distribution of the burrows on the tree, from the small branches less than an inch in diameter and between 40 and 50 feet from the ground down even to the roots, where in one case a larva was found constructing a pupal cell 11 inches below the surface of the ground.

[1] Chittenden, F. H. Loc. cit.

THE PUPA

The pupal stage of the life history has been studied for the most part in the laboratory. During the winter the larvæ were collected in their pupal cells and placed in wash bottles, which were then covered with cheesecloth. The larvæ were found to contract and straighten out in such a way as to face the end of the cell which is next to the bark. When contracted ready for casting the larval skin, the larvæ measured from 6 to 10 mm., instead of 18 to 24 mm., and were greatly swollen, with constrictions marking the posterior limits of the head, prothorax, and the location of the appendages. Two or three days before the larval skin was cast the posterior segments were collapsed and empty. When the pupa was ready, it began a series of wavelike dorsoventral bendings, which caused the skin to break on the dorsal side of the head and prothorax a little to one side of the middle. As these movements continued the skin was slipped gradually backward, collapsing as it left the posterior end of the body, until it was entirely off, when the pupa came to rest (Pl. XXXVIII, fig. 7). The mouth parts passed to the ventral side and seemed to act as a lever against the side of the cell in pushing the skin backward.

The pupal stage lasted about 10 days, during the latter part of which pigmentation began with the eyes, then the mouth parts, head, and thorax. The pupal skin was shed in much the same way as the larval skin, and the adult folded the wings, remaining inactive until the elytra were entirely pigmented. At first the movements of the adults were slow and uncorrelated as contrasted with the great activity later on. The beetle burrowed through the bark as soon as it acquired its full activity and escaped through a characteristic opening (Pl. XXXIX, fig. 2). The openings are always found on the ridges of bark and resemble the shape of the hole made by the larva when it first entered the bark.

Larvæ which had not yet pupated were collected as late as June 17, when adults were found making their way out from the pupal cells. From this it seems that the insect in this state normally pupates during the latter part of May and emerges from the cell about the middle of June.

PARASITIC ENEMIES

Two parasites were incidentally noticed. One, which was reared from the larvæ, was identified by Mr. S. A. Rohwer as a species of the genus Atanycolus, while another, unfortunately mutilated, which was reared from an egg, was placed by Mr. J. C. Crawford in the family Trichogrammidae.

CONTROL OF THE OAK BORER

The method of control heretofore recommended has been the cutting and burning of infested trees before the emergence of the adults in the spring. This is an effective method and needs emphasizing, for people are tempted to leave all the trees which show any signs of life, with the

hope that they will recover the next spring. These are the most dangerous trees, for, as has has been pointed out, the trees with sap are more favorable to the insects than the dry ones in which the larvæ are liable to dry up or starve.

The need of other methods, however, seemed imperative. During the past season the trunks and large limbs of some trees were sprayed with an iron-sulphate and lime-sulphur mixture, while others were sprayed with a Bordeaux mixture. This was done as a preventive measure during the egg-laying season and it seemed successful, as no beetles were seen on the trees which had been sprayed, even though the trees had been covered with beetles the day previous to this treatment. In contrast to this beetles were seen in great numbers throughout the season on the unsprayed trees near by.

Other experiments which are under way can not be reported until at least another season has passed, and greater opportunity has been offered to try out proposed methods of prevention and control.

PLATE XXXVIII

Fig. 1.—*Agrilus bilineatus:* Eggs in position in the bark of an oak tree. ½ natural size.

Fig. 2.—*Agrilus bilineatus:* Cluster of newly laid eggs. ×6.

Fig. 3.—*Agrilus bilineatus:* Eggs shortly before hatching. ×6.

Fig. 4.—*Agrilus bilineatus:* Newly hatched larva. ×6.

Fig. 5.—*Agrilus bilineatus:* Mature larva. ×6.

Fig. 6.—*Agrilus bilineatus:* Larva in its cell. Section made perpendicular to the surface of the bark. *A*, Point at which adult will emerge; *B*, burrow stopped with frass. ×6.

Fig. 7.—*Agrilus bilineatus:* Pupa in cell. Section made parallel to the surface of the bark. ×6.

Fig. 8.—*Agrilus bilineatus:* Adult female. ×6.

Fig. 9.—*Agrilus bilineatus:* Adult male. ×6.

Drawings by Helen A. Sanborn.

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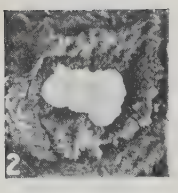

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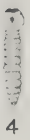

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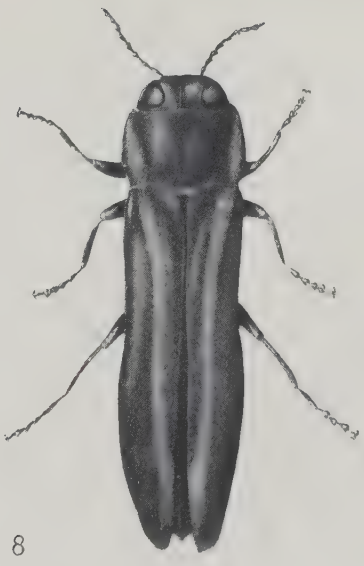

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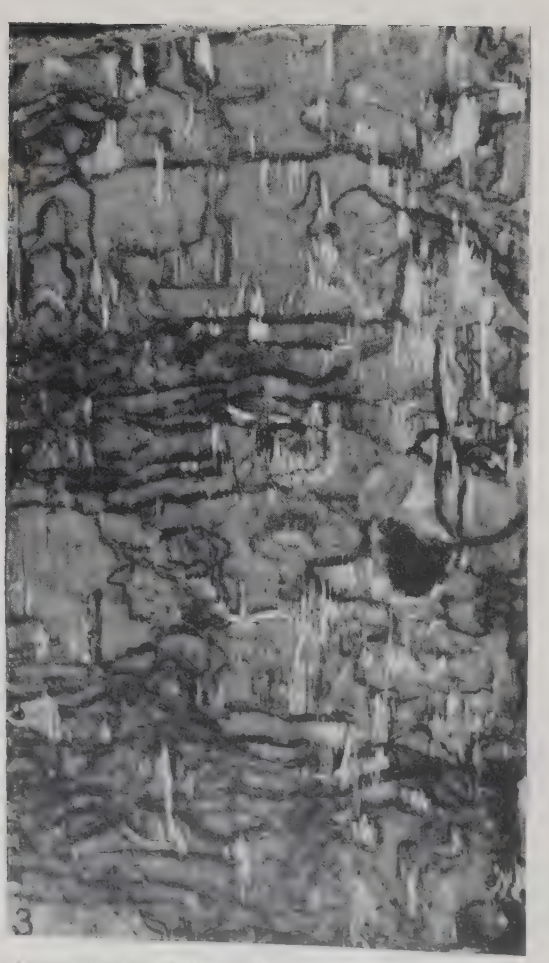

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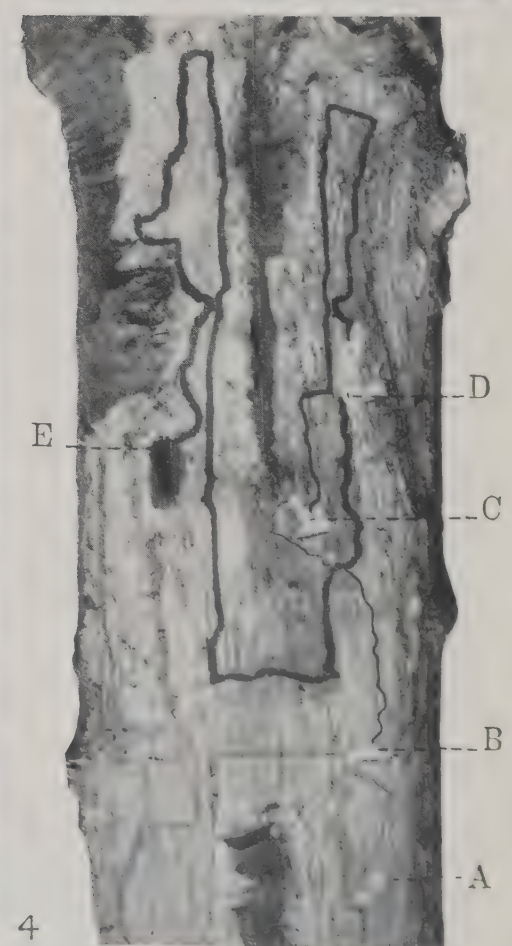

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PLATE XXXIX

Fig. 1.—Leaf showing work of four Agrilus beetles in 24 hours.

Fig. 2.—Hole in bark made by adult Agrilus in emerging from pupal cell.

Fig. 3.—Larvæ of *Agrilus bilineatus* and their burrows.

Fig. 4.—Complete burrow of a larva of *Agrilus bilineatus*. *A*, Point at which larva hatched; *B*, beginning of second instar; *C*, beginning of third instar; *D*, beginning of fourth instar; *E*, pupal cell.

EFFECT OF DILUTION UPON THE INFECTIVITY OF THE VIRUS OF THE MOSAIC DISEASE OF TOBACCO

By H. A. Allard,

Assistant Physiologist, Tobacco and Plant-Nutrition Investigations, Bureau of Plant Industry

In order to obtain some idea concerning the effect of dilution upon the infective power of the virus of the mosaic disease of tobacco in subsequent inoculations, the following experiments were made. A quantity of expressed sap from mosaic-diseased leaves was first passed through filter paper to remove the cell tissue, etc. Clean tap water was then used to bring the filtered virus to the required degree of dilution. All dilutions were accurately determined and inoculations immediately made from these. Young, vigorous plants growing in 3-inch pots in the greenhouse were used in all tests. In order to insure a thorough test of the infectivity of the diluted virus, a drop of the solution carried on the point of the needle was introduced with each puncture. Every leaf of any size on the plants, usually four or five, was inoculated in this manner at several points.

The plants were kept under observation for a long period after the first appearance of the disease in those groups treated with the original undiluted virus and the lower dilutions. This is virtually a quarantine period for the disease, since experience has shown that the incubation period of the mosaic disease is very uniform for simultaneous inoculations under any given set of conditions. A complete tabulation of all dilution experiments is given in Table I.

TABLE I.—*Effect of dilution upon the infectivity of the virus of the mosaic disease of tobacco*

Date of inoculation.[a]	Number of plants.	Variety.	Degree of dilution.	Effect.
1913.				
May 6.....	10	Connecticut Broadleaf.....	Original undiluted virus..	6 mosaic on June 1.
Do.....	10	.....do.....	1 part virus to 100 of water.	7 mosaic on June 1.
Do.....	10	.....do.....	1 part virus to 1,000 of water.	Do.
Do.....	10	.....do.....	1 part virus to 10,000 of water.	4 mosaic on June 1.
Do.....	10	.....do.....	1 part virus to 1,000,000 of water.	All healthy on June 1.
Do.....	10	.....do.....	Tap water alone.....	Do.
May 9.....	20	.....do.....	Original undiluted virus..	13 mosaic on May 26.
Do.....	20	.....do.....	1 part virus to 1,000 of water.	10 mosaic on May 26.
Do.....	20	.....do.....	1 part virus to 1,000,000 of water.	1 mosaic on May 26.
Do.....	10	.....do.....	Tap water alone.....	All healthy on May 26.

[a] All dilutions were prepared on the same day the inoculations were made.

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TABLE I.—*Effect of dilution upon the infectivity of the virus of the mosaic disease of tobacco*—Continued

Date of inoculation.	Number of plants.	Variety.	Degree of dilution.	Effect.
1913.				
May 29	20	Connecticut Broadleaf	Original undiluted virus	11 mosaic on June 5.
Do	19	do	1 part virus to 1,000 of water.	17 mosaic on June 5.
Do	33	do	1 part virus to 1,000,000 of water.	All healthy on June 5.
Do	10	do	Tap water alone	Do.
June 12	15	do	Original undiluted virus	11 mosaic on June 18.
Do	21	do	1 part virus to 1,000 of water.	8 mosaic on June 18.
Do	21	do	1 part virus to 10,000 of water.	7 mosaic on June 18.
Do	30	do	1 part virus to 1,000,000 of water.	1 mosaic on June 18.
Do	10	do	Tap water only	All healthy on June 18.
June 21	30	20 Cuban 10 Connecticut Broadleaf	Original undiluted virus	26 mosaic on July 6 (18 Cuban, 8 Connecticut Broadleaf).
Do	30	15 Cuban 15 Connecticut Broadleaf	1 part virus to 1,000 of water.	20 mosaic on July 6 (11 Cuban, 9 Connecticut Broadleaf).
Do	33	18 Cuban 15 Connecticut Broadleaf	1 part virus to 10,000 of water.	12 mosaic on July 6 (4 Cuban, 8 Connecticut Broadleaf).
Do	20	10 Cuban 10 Connecticut Broadleaf	Tap water only	All healthy on July 6.
June 24	12	Cuban	Original undiluted virus	12 mosaic on July 6.
Do	20	do	1 part virus to 1,000 of water.	19 mosaic on July 6.
Do	17	do	1 part virus to 10,000 of water.	10 mosaic on July 6.
Do	21	do	1 part virus to 100,000 of water.	2 mosaic on July 6.
Do	10	do	Tap water only	All healthy on July 6.
Do	75	do	Not inoculated	Do.
1914.				
April 2	10	Connecticut Broadleaf	Original undiluted virus	6 mosaic on April 17.
Do	10	do	1 part virus to 1,000 of water.	2 mosaic on April 17.
Do	10	do	1 part virus to 10,000 of water	1 mosaic on April 17.
Do	10	do	1 part virus to 100,000 of water.	Do.
Do	20	do	1 part virus to 1,000,000 of water.	All healthy on April 17.
Do	10	Nicotiana rustica	Original undiluted virus	4 mosaic on April 17.
Do	10	do	1 part virus to 1,000 of water.	Do.
Do	10	do	Tap water alone	All healthy.
April 3	10	Connecticut Broadleaf	Original undiluted virus	3 mosaic on April 17.
Do	10	do	1 part virus to 1,000 of water.	5 mosaic on April 17.
Do	10	do	1 part virus to 1,000,000 of water.	All healthy on April 17.
Do	10	do	Tap water alone	Do.
Do	10	Nicotiana rustica	Original undiluted virus	8 mosaic on April 17.
Do	10	do	1 part virus to 1,000 of water.	7 mosaic on April 17.
Do	10	do	do	2 mosaic on April 17.
Do	10	do	Tap water alone	All healthy on April 17.
April 9	10	Connecticut Broadleaf	Original undiluted virus	9 mosaic on April 25.
Do	10	do	1 part virus to 1,000 of water.	7 mosaic on April 25.
Do	10	do	1 part virus to 5,000 of water.	2 mosaic on April 25.
Do	10	do	1 part virus to 10,000 of water.	Do.
Do	10	do	1 part virus to 20,000 of water.	All healthy on April 25.
Do	10	do	1 part virus to 50,000 of water.	1 mosaic on April 25.
Do	10	do	1 part virus to 100,000 of water.	All healthy on April 25.
Do	10	do	1 part virus to 200,000 of water.	Do.
Do	10	do	1 part virus to 500,000 of water.	Do.

TABLE I.—*Effect of dilution upon the infectivity of the virus of the mosaic disease of tobacco*—Continued

Date of inoculation.	Number of plants.	Variety.	Degree of dilution.	Effect.
1914.				
April 9.....	10	Connecticut Broadleaf	1 part virus to 1,000,000 of water.	All healthy on April 25.
Do.....	10	.....do.....	Tap water alone.....	Do.
Do.....	10	.....do.....	Not inoculated	Do.
Do.....	10	Maryland Mammoth.....	Original undiluted virus...	9 mosaic on April 25.
Do.....	10	.....do.....	1 part virus to 1,000 of water.	6 mosaic on April 25.
Do.....	10	.....do.....	1 part virus to 5,000 of water.	5 mosaic on April 25.
Do.....	10	.....do.....	1 part virus to 10,000 of water.	2 mosaic on April 25.
Do.....	10	.....do.....	1 part virus to 20,000 of water.	All healthy on April 25.
Do.....	10	.....do.....	1 part virus to 50,000 of water.	Do.
Do.....	10	.....do.....	1 part virus to 100,000 of water.	Do.
Do.....	10	.....do.....	1 part virus to 200,000 of water.	Do.
Do.....	10	.....do.....	1 part virus to 500,000 of water.	Do.
Do.....	10	.....do.....	1 part virus to 1,000,000 of water.	Do.

These tests show beyond question that the virus of the mosaic disease when diluted to 1 part in 1,000 of water is quite as effective in producing infection as the original undiluted virus. A dilution of 1 part in 10,000, however, gives evidence af attenuation. At greater dilutions than this the chances of infection are very greatly reduced. In dilution experiments of this sort, where increasing attenuation of the virus is taking place, it is obvious that no sharp line of demarkation can be found beyond which chances of infection do not exist. Since the solutions are punctured into the leaves with a sharp needle, the quantity of virus taken up and actually introduced into the plant tissues must be exceedingly small, especially for the higher dilutions. It is of interest to consider this fact in connection with the enzymic theory of the mosaic disease, which has been advanced to explain the nature of the disease. This theory assumes that the mosaic disease devolops when certain oxidizing enzyms normally present in the plant increase in amount or in activity as a result of various external conditions affecting nutrition and growth.

It is somewhat difficult to reconcile this theory with the fact that a tiny drop of virus diluted to 1 part in 10,000 can readily produce the mosaic disease. It must be assumed that the immeasurably small quantity of oxidizing enzyms carried by this drop is sufficient to increase the normal oxidase content already present in the plant to the extent of a permanent pathological reaction resulting in the mosaic disease. This is highly improbable, since it is well known that the oxidase content of healthy individuals normally varies to a measurable degree in response to various environmental changes.

There seems to be no logical reason for considering that something exists in the constitution of all normal tobacco plants which is always capable of producing the mosaic disease in response to suitable conditions. This conception does not harmonize with the fact that even when the virus of the mosaic disease is highly diluted and the infective substance becomes immeasurably small it is still capable of initiating the disease when introduced into healthy plants. All evidence at hand points to something in the virus quite extraneous to the protoplasmic constitution of healthy plants. Once introduced into the tissues of such plants, this foreign substance rapidly increases in quantity and becomes actively prejudicial to those physiological activities associated with normal nutrition and growth.

In the opinion of Woods and Heintzel this substance constituting the active, pathogenic principle of the virus of the mosiac disease may be regarded as purely chemical, nonliving, enzymic, and a normal constituent of all healthy tobacco plants. According to Hunger, on the other hand, the disease is caused by a toxic ferment not normally present in the cells of healthy plants, but which develops in response to unfavorable conditions of nutrition and growth. These theories are in complete agreement in ascribing to the mosaic disease a spontaneous origin within susceptible plants under favorable conditions. The development of this conception is quite natural if a spontaneous origin is accepted, since this does not admit of a consistent explanation on the basis of parasitism. At the time these theories were evolved, little was known concerning organisms which are smaller than the visible bacteria and yet responsible for parasitic diseases. It was known that the visible bacteria could not pass through the pores of certain filters. It was also discovered that passing the virus of certain diseases through these filters did not necessarily deprive it of its power to infect, although visible parasites were no longer present. At this time this characteristic seemed to remove those diseases connected with a filterable virus from that class of diseases definitely established as bacterial in their origin. Until additional facts had been secured an enzymic origin was perhaps the most plausible explanation for those obscure diseases connected with a filterable virus and supposedly capable of a spontaneous origin within certain plants.

The writer's experiments, however, indicate that the mosaic disease can not be induced to arise spontaneously in healthy plants by the operations of cutting back, repotting, or otherwise subjecting the plants to unfavorable conditions.

SUMMARY

The virus of the mosaic disease when diluted to 1 part in 1,000 of water is quite as effective in producing infection as the original undiluted virus. Attenuation of the virus is indicated in dilutions of 1 part in 10,000 of water. At greater dilutions infection is not likely to occur.

The virus of the mosaic disease is highly infectious to all susceptible, healthy plants. Such plants remain free from this disease so long as all chances of accidental infection are excluded. All evidence at hand indicates that something is present in the virus of the mosaic disease which is extraneous to the protoplasmic organization of healthy plants. This substance greatly increases in quantity when introduced into susceptible plants and interferes with normal nutrition and growth.

Although enzymic activities have been considered responsible for the mosaic disease of tobacco, parasitism, in the writer's opinion, offers by far the simplest and most reasonable explanation of its origin. It may at least be said that the theory of a parasitic origin for the disease more consistently accounts for all the facts at hand than any enzymic conception yet evolved. It seems not only needless but illogical to abandon a simple, direct explanation for one which leads to complexity of thought and yet fails to correlate all the facts at hand.

MOLDINESS IN BUTTER

By CHARLES THOM, *Mycologist*, and R. H. SHAW, *Chemist, Dairy Division, Bureau of Animal Industry*

INTRODUCTION

References to mold in butter are not uncommon in dairy literature, but specific information is lacking as to what forms of mold occur on butter, the conditions which permit mold development, and the actual changes produced in the butter. As met in the market, losses from mold take two forms: (1) The growth of mold upon the tub, lining, or wrapper injures the appearance and salability of the package without seriously affecting the quality of its contents. (2) Mold development upon the butter itself when continued for a considerable period produces changes which can not be eliminated even by the renovation process. Such butter becomes an actual loss. The work reported here aims to cover the biological phases of this problem. The study of the chemical changes produced in the butter will be reported later.

ORIGIN OF BUTTER SAMPLES

Characteristic samples representing the range of conditions and appearances found in commercial butter were obtained through the inspection service of the Dairy Division. These were examined in the mycological laboratory. The number and variety of mold colonies upon each sample were noted and cultures were made to obtain the species represented. The samples were then taken to the chemical laboratory for analysis. Consideration of the known factors influencing mold growth called for the determination of the quantities of water and protein available, together with the percentage of salt as a possible limiting factor.

In Table I are given the analyses of samples of moldy butter from several sources.

TABLE I.—*Analyses of samples of moldy butter*

Sample No.	Water.	Salt.	Curd.[a]	Sample No.	Water.	Salt.	Curd.[a]
	Per cent.	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*
3515	11. 65	1. 98	1. 48	3554s	7. 38	0. 63	0. 57
3529	12. 00	. 65	1. 53	3546a	16. 02	3. 05	. 68
3530	9. 40	2. 19	. 64	3546b	18. 00	1. 40	. 75
3554g	11. 09	2. 66	. 66	3546c	16. 02	3. 50	. 62
3554r	11. 72	2. 13	. 64				

[a] The term "curd," as used in this paper, means the amount of nitrogen multiplied by the factor 6.38.

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Samples Nos. 3546a, 3546b, and 3546c were taken from a tub of butter containing three small churnings. The tub had been kept in a refrigerator for three or four months and was very rancid. Since the butter was designed for packing stock to be used in experimental work on renovated butter, no particular care was taken in its manufacture. It happened that the top layer (3546a) and bottom layer (3546c) were heavily salted, while the middle layer (3546b) contained but a small percentage of salt and a considerably higher percentage of water. The top and bottom layers were free from mold; the middle layer showed areas typically representing each of the types of moldiness described in the following pages.

TYPES OF MOLD FOUND IN SAMPLES

From the study of these and other available samples of moldy butter three well-marked types of mold effects are distinguished:

1. SMUDGED, OR ALTERNARIA, TYPE.—In samples Nos. 3515, 3546b, and 3554s, dark, smoky, or rarely greenish colors occurred in patches which suggested soot or dirty-finger marks. Microscopic examination showed mold mycelium with dark-brown or green walls on or under the surface. Frequently these colonies are entirely submerged in the butter. Sometimes hyphæ were observed 4 to 5 mm. below the surface. Spores were rarely found, but colonies transferred to culture media grew freely and fruited normally. The dark-brown or black hyphæ were the most common and proved to be species of Alternaria. Where a greenish color was seen species of Cladosporium developed. These submerged areas suggest the appearances noted by Patterson (1900),[1] and attributed to *Stemphylium butyri* Patterson. The occurrence of Cladosporium in butter has been studied by Jensen (1900) and the organism found was named by him "*Cladosporium butyri.*" The species of Cladosporium, however, are abundant upon all kinds of roughage fed to cows, and the spores find entrance to the milk from the handling of such feed by the milkers. One of the writers had access to cultures made from many samples of cream by the bacteriologists of the Storrs Agricultural Experiment Station some years ago. In these cultures colonies of Cladosporium were so abundant as to indicate that spores of these species remain with the cream after separation. Species of Alternaria are very common in the same circumstances and appeared in these cultures, but less abundantly. Their appearance as colonies in the butter, therefore, is due to the ability of these species to grow in the very severe conditions imposed by a mass of butter. One of the writers has found a species of Alternaria growing and fruiting in a box of shoe paste. Species of this group have also been isolated from various forms of fat when small inclusions of water occur. Few other graminicolous fungi seem able to produce colonies under these conditions, though spores of many kinds are undoubtedly present.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 310.

In at least one sample, contributed by Dr. G. P. Clinton, of the Connecticut Agricultural Experiment Station, and again in a sample of fat studied by Dr. C. N. McBryde, of the Bureau of Animal Industry, a fungus producing abundant orange to red mycelium and red blotches upon the butter was obtained. In butter and in the culture media used no spores have thus far been obtained. This organism grows under the same conditions as Alternaria.

2. GREEN-MOLD TYPE.—Green molds were found more or less frequently upon all the samples tabulated except Nos. 3546a and 3546c. Cultures of these molds proved to be species of Penicillium. Three common species were often found. These were *P. roqueforti*, a variety or strain of *P. expansum*, and *P. chrysogenum*. Several other forms difficult to identify were occasionally obtained. Aside from *P. roqueforti*, these are identifiable only by careful culture and comparison. These molds form green patches on the surface and follow seams or cracks into the mass. In one tub (No. 3515), where extensive moldy areas were found in cracks and seams, the presence of *P. roqueforti* was suggested by a strong odor and flavor resembling that of Roquefort cheese. Marked physical changes in the fat itself were noticeable. Culture confirmed the identification of the organism. So far as observed, no such extensive changes were produced by the other species. The storage of butter in tubs is accompanied by low percentages of free oxygen[1] in the butter suggestive of the conditions in Roquefort cheese (Thom and Currie, 1913). Mold is found upon the liners, upon the inside of the tub itself, and in the cracks of the butter. In all these places interchange of gases is very slow, thus favoring the dominance of Roquefort mold, which is more tolerant of such conditions than other species.

3. OIDIUM TYPE.—The third form produces various shades of orange-yellow discoloration, with little or no surface growth. Culture and microscopic examination show that these areas are produced by *Oidium lactis*. This organism grows to the depth of several millimeters within the mass of butter as a complex mycelium with hyphæ varying in diameter with the size of the spaces between the masses of fat. Some spores are formed and at times surface-fruiting areas. Bacterial activity is commonly associated with the presence of this mold.

BLACK MOLDS, OR MUCORS.—Where butter has been moist enough for loose masses of surface mycelium to grow, mucors are sometimes seen. These molds are found by culture to be present in many other samples in which no visible colonies are produced.

EXPERIMENTAL WORK

To study the conditions favorable to mold growth in butter special samples of butter were prepared, some low in water content and some high in water content, some thoroughly washed to reduce the curd con-

[1] Unpublished results of Dr. D. C. Dyer, of the Dairy Division.

tent and some with casein added to raise the protein content. One ounce of salt to 1 pound of butter was used in some samples; no salt in others. Slices of butter of each kind were put into Petri dishes and inoculated with a series of molds obtained from butter. Among these were *Oidium lactis*, *Mucor* sp., *Alternaria* sp., and several species of Penicillium. The dishes were then allowed to stand in an incubator at the temperature and relative humidity of the laboratory for several days. Absolutely no surface growth of mold was obtained. Part of these Petri dishes were then placed in moist chambers and it was found that mold colonies developed upon every sample so placed. These growths included not only the species inoculated into the butter but other forms whose spores were present in the butter as made. At the low relative humidities prevailing in the laboratories of the Dairy Division from February to April, 1914, no mold colonies were able to develop in butter representing a range in water content greater than the usual range of percentage in market butter.

The addition of 2 to 3 per cent of water to butter containing but 14 or 15 per cent does not make the quantity of water present sufficient to support mold growth aside from conditions of high humidity.

RELATION OF HUMIDITY TO MOLD GROWTH

In moist-chamber culture comparison between samples containing normal and low protein with samples containing excess or added protein showed that mold growth was more rapid and extensive when protein was added. The failure of molds to grow in these same cultures under the ordinary humidity conditions of the laboratory proved that the essential factor in molding was not protein, but water.

To define these humidity relations more closely, three desiccators were prepared in which definite relative humidities could be maintained. For this purpose the bases of the desiccators were filled with sulphuric acid standardized to the specific gravities—from Hastings's (1909) table—required to maintain, respectively, 90 per cent, 79.6 per cent, and 69.6 per cent relative humidity. Three samples were used: One sample of butter was made with low-salt content (0.55 per cent); one at normal salting (2.43 per cent); and one sample of butter fat, free from water, with skim-milk powder added.

The composition of these three samples is given in Table II.

TABLE II.—*Composition of samples of butter used in mold-growth tests*

Character of sample.	Water.	Salt.	Curd.
	Per cent.	*Per cent.*	*Per cent.*
Normal-salt butter	15.2	2.43	0.62
Low-salt butter	15.6	.55	.62
Butter fat + dry skim milk	None.	None.	.48

Three slices, one from each of these samples, were put into each one of a series of 24 Petri dishes. The three slices in each dish were thus under absolutely the same conditions. Six species of mold were then selected and were heavily inoculated into the plated slices—each slice in four Petri dishes being inoculated with one species of mold. Four sets of six dishes each were thus available. One set of six Petri dishes was put into a moist chamber (approximately 100 per cent of relative humidity), and one each into the desiccators at 90, 79.6, and 69.6 per cent relative humidity. The cultural results are given in Table III.

TABLE III.—*Effects of salt and humidity on mold growth in butter*[a]

Mold.	Growth in moist chamber.			Growth under relative humidities of—								
				90.6 per cent.			79.6 per cent.			69.6 per cent.		
	Salted butter.		Butter fat and curd.	Salted butter.		Butter fat and curd.	Salted butter.		Butter fat and curd.	Salted butter.		Butter fat and curd.
	0.55 per cent.	2.43 per cent.		0.55 per cent.	2.43 per cent.		0.55 per cent.	2.43 per cent.		0.55 per cent.	2.43 per cent.	
Alternaria sp	0.9	0	0	0.3	0	0	0.6	0	0	0	0	0
Mucor sp	(?)	0	0	0	0	0	0	0	0	0	0	0
Oidium sp	[b]0.6		0	.5	0	0	.5	0	0	.3	0	0
Penicillium roqueforti	.5	0	0	.5	.2	0	0(?)	0	0	0(?)	0	0
Penicillium chrysogenum	.5	.1	.8	.4	.2	.3	.3	0	.2	.3	0	0
Penicillium expansum	.5	.1(?)	0	.3	0	0	.3	0	0	0	0	0

[a] A typical colony would be designated as 1.0; lesser growths by decimal fractions.
[b] Submerged.

Examination of this table shows that a single species, *Penicillium chrysogenum*, was able to produce a colony upon the butter fat plus the water obtainable from the air. Careful examination of the other samples showed no mold. In the low-salted butter, however, with 15.6 per cent of water marked growth occurs; the water in this butter is therefore to be regarded as an essential factor in the molding found here. The butter containing 2.43 per cent of salt shows determinable growth from but two species, *P. roqueforti* and *P. chrysogenum*. No growth of species of Alternaria, Oidium, or Mucor was found upon this butter. The low-salted butter shows very appreciable mold colonies of all species except the Mucor. Growth was greatest in the moist chamber. Nearly as good growth was obtained, however, with a relative humidity of 90.6 per cent, and considerable growth in four of the species with 79.6 per cent. In the presence of 69.6 per cent there was very little visible mold, even in the low-salted sample. The individual samples of low-salted butter all showed the characteristic orange-yellow colors due to the development of spores of *Oidium lactis*, which were evidently present from the first in all slices. In this experiment the organism grew only in its submerged form; hence, it was little affected by the relative humidity to which the other species responded so clearly. Alternaria and Oidium developed

only in the low-salted samples. Alternaria appeared in several places without inoculation. *Rhizopus nigricans* was found once in a moist-chamber sample. *Aspergillus fumigatus* and *A. niger* both appeared in one or more cases, but none of these species appeared where the percentage of salt was 2.43.

The same fact is illustrated on a larger scale by the tub of butter analyzed as No. 3546 in Table I. Of the three samples packed together in one tub the middle layer was low-salted and typically moldy, while the top and bottom layers were free from mold.

These results harmonize fully with the data from the analysis of butter as found in Table I and with the preliminary cultural data as given in subsequent experiments. Two of the three types of moldiness, the smudged and the orange-yellow forms, occur only in butter containing less than 2 per cent of salt. Even with green molds under high humidities and at temperatures far above those used in storage, growth in these experiments was negligible in butter with a salt content of 2.43 per cent.

THE SALT FACTOR IN MOLD GROWTH

The salt factor in butter is calculated as follows: Thompson, Shaw, and Norton (1912), in analyzing 695 samples of American creamery butter, found an average water content of 13.9 per cent; salt content, 2.51 per cent; and curd content, 1.18 per cent. This amount of salt in solution in the water present forms, therefore, approximately a 13 per cent brine, which represents the brine formed by adding 18 parts of salt to 100 parts of water. If the same water content be assumed and the salt content found be 1 per cent, the brine present is 5.1 per cent (made by adding 7.1 parts of salt to 100 parts of water); with a salt content of 2 per cent, this strength would be 10.2 per cent; with 3, 15.3 per cent; and with 4, 20.4 per cent. For purposes of mold growth the strength of the brine found is one very significant factor. Another factor is represented by the distribution of air and moisture throughout the mass of butter, and still a third by the relative humidities to which the butter is subjected.

To obtain more complete cultural data for comparison, a series of cultures was made with media containing known percentages of salt. For this purpose 6.5 per cent of salt was introduced into one lot of Czapek's agar (Dox, 1910) and 14 per cent in a second lot. The first represents approximately the proportion of brine in butter with 1.3 per cent of salt, the second the brine with a content of about 2.8 per cent of salt. These cultures were grown in a moist chamber to eliminate the concentration of the brine by drying. The cultural results are given in Table IV.

TABLE IV.—*Cultural results with media containing known percentages of salt*[a]

Mold.	Percentage of salt.		Mold.	Percentage of salt.	
	6.5	14.4		6.5	14.4
Alternaria sp. 3515	0.7	0.2	Penicillium roqueforti 3515C	1.0	[b] 1.0
Alternaria sp. 3513	.7	.2	Penicillium expansum.	.9	.4
Alternaria sp. 3546	.7	.2	Penicillium stoloniferum, var	1.0	[b] .7
Cunninghamiella sp	.6	0	Penicillium chrysogenum	.9	.7
Fusarium sp	.4	0	Penicillium purpurogenum		0
Mucor sp. 3513	.7	0	Red mold 3536.3	.3	0
Mucor sp. 3514D4	.6	0	Rhizopus nigricans	.7	0
Mucor sp. 3514C1	.6	0	Trichoderma sp	.3	0
Mucor sp. 3532	.5	0			
Oidium lactis	.1	0			
Penicillium sp. 3529a	1.0	[b] 1.0			
Penicillium roqueforti	.9	[b] .4			

[a] A typical colony is designated as 1.0; lesser growth by decimal fractions.
[b] These cultures developed slowly, but finally reached the condition indicated.

These cultural results agree with other data published recently (Thom, 1914). Two more series of cultures were made, containing approximately 18 and 21 per cent of salt. In these such organisms as produced marked growth with a salt content of 14.4 per cent were carried, together with other species of Penicillium and Aspergillus. *Penicillium chrysogenum*, *P. stoloniferum*, *Penicillium* sp. 3529a and *Aspergillus repens* produced considerable growth with 18 per cent of salt. Three other organisms produced slight growth. With 21 per cent of salt no colonies were obtained, although spores of *P. chrysogenum* germinated. Comparison with the results of butter inoculation shows that Czapek's solution sustained much larger growth than butter containing comparable percentages of salt. To show the results of these culture series for the organisms obtained from butter, the graphic representation (fig. 1) was prepared. The four series reported were calculated as representing approximately brine conditions in butter containing 1.3, 2.7, 3.4, and 4.1 per cent of salt. Even under the very favorable conditions offered by the culture media, temperature, and humidity used, the mold growth found in the second series was small and in the third series was negligible.

DISCUSSION OF RESULTS

From the data already given, mold is seen to attack the butter itself if unsalted or very lightly salted. Normally salted butter may be affected by green mold only if held under conditions very favorable to mold growth. In general such losses are not great. Both the species of Oidium with its orange-yellow patches and the smudges of Alternaria disappear promptly when even very moderate salting is practiced. These are the important factors in losses of unsalted butter as studied by Jensen (1901 and 1908). Since *Oidium* sp. penetrates the mass of butter

and produces marked discoloration, as well as bad flavors, salting, if practiced at all, should be heavy enough to eliminate this group of organisms. Green molds may damage normally salted butter if cracks and open spaces are left by bad packing. In most cases such mold will be confined to liners and containers if the packing is fairly well done. Rogers (1906) found that paraffining the tubs or boxes used prevented mold on both container and liner. The paraffin prevented the escape of water which would leave the air spaces necessary for mold growth, thus

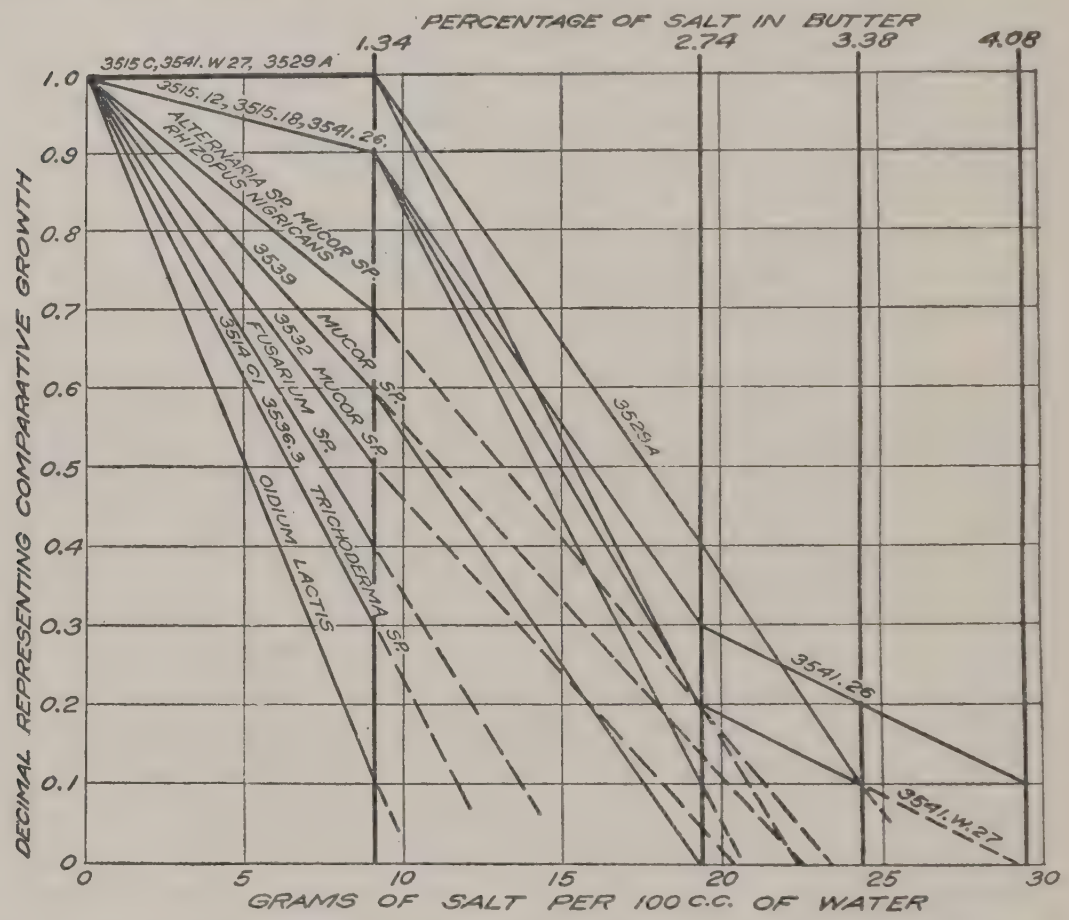

FIG. 1.—Graph showing the effect of salt on molding. Cultural results with organisms obtained from butter. Nos. 3515c, 3541W27, 3929a, 3515.12, 3515.18, and 3541.26 were species of Penicillium. No. 3514C1 was a species of Mucor and No. 3536.3 was the sterile red mold from butter, The dotted portions of the graph represent hypothetical courses for organisms disappearing at percentages not determined but limited by the next experiment.

preventing also loss of weight from the butter itself. Previous papers have taken no account of the presence of mold spores in the butter itself. All possible treatment of containers will fail unless conditions are produced which will prevent the growth of these spores. The same conditions which stop the growth of molds present on the paper and wood of the package also prevent the spores in the butter from growing.

In all storage of butter the temperature factor must not be neglected. Mold growth is progressively reduced by low temperatures. Work elsewhere reported shows that species of Penicillium (Thom, 1910, p. 92, 93, 105) grow very slowly as the temperature approaches the freezing point.

If, as in butter, the fluid present is a strong brine, the temperature must be actually carried considerably below the freezing point of water to eliminate danger from the growth of micro-organisms. Temperatures a few degrees above freezing accompanied, as they frequently are, by moist conditions are favorable to molding in butter. Unsalted butter is more subject to deterioration from micro-organisms than salted butter. Successful storage of such butter is therefore even more dependent upon scrupulously clean dry refrigeration at low temperatures than is the case with salted butter. Cellars and ice refrigeration rarely furnish conditions which will prevent mold growth in unsalted or low-salted butter, although such growth may be delayed or reduced. Butter properly made and salted normally, as indicated above, will not show mold under reasonably careful handling.

SUMMARY

(1) Mold in butter usually takes three forms:

a. Orange-yellow areas with a submerged growth of mycelium are produced by *Oidium lactis.*

b. Smudged or dirty-green areas either entirely submerged or with some surface growth are produced by species of Alternaria and Cladosporium.

c. Green surface colonies are produced by species of Penicillium, or, more rarely, Aspergillus, either upon the butter, causing decomposition, or upon the container or wrappings, injuring the appearance of the sample in the market.

(2) Species of Oidium, Alternaria, and Cladosporium can not develop in butter containing 2.5 per cent of salt. The occurrence of any of these forms in a sample of butter indicates low salting.

(3) Excess of curd favors mold growth. Well-washed butter is less subject to mold.

(4) Leaky butter—butter from which water of buttermilk exudes and collects in the wrappings or in the container—furnishes the best conditions for the beginning of mold growth. From these wet areas colonies may spread to the butter itself.

(5) Wet surfaces, wet wrappings, or high humidity are essential to mold growth in butter. Mold will not grow upon the surface of a piece of butter exposed to humidities of 70 per cent or lower. The water in the butter is thus not sufficiently available to the mold to support the development of a colony, unless evaporation is reduced by high humidities. In closed packages, wet or damp cellars, or carelessly packed masses with cracks or fissures in which moisture collects, mold may seriously injure the appearance of butter packages or actually induce great changes in the butter itself.

(6) Salt up to 2.5 to 3 per cent in butter is sufficient to eliminate mold or reduce it to negligible amount. This is equivalent to the use of a 12 to 15 per cent brine.

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SUSCEPTIBILITY OF CITROUS FRUITS TO THE ATTACK OF THE MEDITERRANEAN FRUIT FLY

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INTRODUCTION

Since the discovery in 1910 that the Mediterranean fruit fly (*Ceratitis capitata* Wied.) had become established in the Hawaiian Islands, the fruit growers, and especially the citrous fruit growers, of the mainland States have increasingly feared that this dreaded pest would be able to gain access to the mainland on some one of the many ships plying between Honolulu and the Pacific coast and would appear in the citrous orchards of California and Florida. In addition to this danger from the Pacific, there have been similar fears regarding imported fruits from the Bermudas and the Mediterranean regions. While investigations carried on by the Federal Horticultural Board have shown that the opportunity for entry and establishment of the fly from these trans-Atlantic countries is very slight, there remains the ever-present danger that sooner or later this pest will reach the mainland from the Pacific, in spite of the increasingly rigid quarantine of Hawaiian host fruits. It is therefore opportune to record data secured in the Hawaiian Islands which tend to show that even if this fruit fly should obtain a foothold in the warmer portions of the United States, it probably would not be the serious pest to citrous fruits that previously published literature would indicate.

HISTORICAL REVIEW

This literature has been full of references to the havoc caused to citrous fruits by the Mediterranean fruit fly. The first published reference is by Latreille, who states (1817) [1] on the authority of Cattoire that the colonists of Mauritius could with difficulty obtain citrous fruits sound at maturity, on account of the attacks of a dipterous insect that deposited eggs in the fruit. MacLeay (1829) writes of this pest as an insect very destructive to oranges and states that fully one-third of the oranges arriving in London from the Azores were in a decayed condition as a result of the attacks of this pest. He also secured the insect from citrous fruits in Madeira and the Cape Verde Islands. F. DeBreme (1842) speaks of this fruit fly as a pest to oranges near Malaga, Spain; and Westwood (1848), under the caption "The Orange Fly," mentions securing specimens from

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 330.

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decayed oranges received at London from St. Michael. Villeneuve (1859) exhibited before the Entomological Society of France an infested orange from Algeria, and Laboulbène (1871) describes the injuries caused by the fruit fly to oranges in Algeria and quotes from notes furnished him by Boisduval to the effect that at Bildah and in all Algeria the orange crop was completely destroyed by the insect. On the other hand, Rondani (1870) writes that the species is rare in Spain and is found in Italy only in the southern part.

While the purpose of this article is not to record the literature of this fruit fly, these few references are sufficient to show that much of the early literature greatly emphasizes the destructiveness of the Mediterranean fruit fly to citrous fruits and has laid little stress upon other fruits more susceptible to attack. It is also interesting to note that much of this older literature, which has been generously copied by later writers, records damage to citrous crops grown in very equable climates and in localities where presumably, as in the Hawaiian Islands, there are many host fruits whose commercial value was so small that they escaped the notice of these writers, who judged of the seriousness of the pest by the fruits arriving at their home markets or from common reports. It is also very possible, with our more exact knowledge of the causes of the decay of fruit in transit and of the wholesale shedding of citrous fruits in the field, due to several fungous diseases, to question the reliability of some of the earlier statements.

HOST FRUITS

Apparently the first observer who did not entirely agree with MacLeay's statement that whenever a puncture is found in the rind of the orange "there is a worm concealed in the interior" is Laboulbène, who said that when he compared his observations on the damage done to oranges by the Mediterranean fruit fly with those recorded by others he found certain contradictory facts which needed further investigation. These contradictory facts, although Laboulbène did not know it, were concerned with what has been determined by the writers as an excessive mortality occurring among the eggs and larvæ of the Mediterranean fruit fly in the orange rind. This mortality, which in the examination of 39 grapefruit that were yellow in color amounted to 99.7 per cent of 7,722 forms, as shown in Table I, will prove a very effective factor in checking this pest in the citrous regions of the United States, especially when combined with the climatic and floral characteristics of these citrous regions and the method of growing and harvesting the fruit.

TABLE I.—*Results of examinations of ripe citrous fruits infested by the Mediterranean fruit fly*[a]

Kind of fruit.	Number of fruits examined.	Punctures.		Eggs.		Larvæ.						Total number of forms examined.
						Alive.			Dead.			
		Empty.	Not empty.	Normal.	Abnormal.	In puncture.	In rag.	In pulp.	In puncture.	In rag.	In pulp.	
Grapefruit	39	123	378	959	5,882	20	1	0	534	326	0	7,722
Lemon	50	380	185	693	729	29	0	0	339	15	0	1,805
Lime	50	180	218	345	187	0	0	25	474	424	0	1,455
Shaddock No. 1	14	0	44	5	36	0	152	55	17	38	0	303
Shaddock No. 2	14	48	237	6	38	5	2	3	405	696	0	1,155
Kusaie lime	17	194	80	196	201	0	5	0	156	280	0	838
Sweet orange	58	251	452	287	397	55	0	17	1,237	1,652	0	3,645
Sour orange	28	57	174	664	1,026	20	9	231	347	59	1	2,357
Chinese orange	85	1	115	207	286	0	8	383	8	14	17	923

[a] These examinations were made sufficiently long after the fruits were gathered to permit all eggs to hatch. All eggs recorded in tables are in reality dead, even though certain of them are marked "normal" in appearance.

The excessive mortality referred to does not mean that citrous fruits are less attractive to the adult Mediterranean fruit flies or that the fruit of certain species of the Citrus family is not capable of becoming badly infested. Reference to Table I shows that the female fly freely oviposits in grapefruit, lemons, limes, shaddocks, and sweet, sour, and Chinese oranges. Whatever may be the degree of preference shown by the females for other fruits, it is not great enough, at least under Hawaiian conditions, to lead them entirely to ignore citrous fruits, even when these are grown in close proximity to such a favored host fruit as the peach. A study of the data in Table II shows that the female has a much stronger preference for the mango (*Mangifera indica*) and the ball kamani (*Calophyllum inophyllum*) than she has for the orange or lemon. While the data are very limited as to the amount and the number of fruits treated, they are indicative of conditions in the field covering a larger range of fruits. In Bermuda during December, 1913, the senior writer found oranges unaffected while Thevetia and loquats (*Eriobotrya japonica*) were well infested. Unfortunately for experimental purposes there are in Hawaii no large Citrus orchards free from other host fruits. Instead there are growing a great profusion of host fruits, chiefly in city or suburban districts, which furnish a rapid succession of fruit flies. No matter, therefore, what preference the ovipositing females may show for noncitrous fruits, the flies are present in such large and constantly augmented numbers that the slowly maturing citrous fruits are bound to be attacked. This is especially true during the months of December, January, and February, when a comparatively small number of host fruits other than Citrus are in season. Like conditions also exist at other seasons of the year during the short intervals between the ripening of other host fruits. While many of these host fruits ripen quickly, the citrous fruits, with the exception of the Chinese orange, develop

slowly and offer themselves for attack over a considerable length of time. Female fruit flies have been seen in Honolulu ovipositing in certain grapefruits and oranges over a period of two or three months. It is not therefore contradictory to the statement that citrous fruits are not the preferred hosts of the fly that we find so large a number of punctures recorded in Table I.

TABLE II.—*Host-fruit preference of the Mediterranean fruit fly*

Experiment No.	Combination and condition of fruits.	Number of punctures.	Number of eggs.
1	Orange, ripe but green in color	5	17
	Mango, partially ripe	10	92
2	Orange, partially ripe	6	22
	Mango, partially ripe	18	101
3	Orange, ripe	16	105
	Mango, ripe	15	135
4	Orange, partially ripe	9	23
	Mango, partially ripe	8	84
5	Orange, ripe	0	0
	Mango, ripe	2	10
6	Lemon, green in color	0	0
	Mango, partially ripe	5	53
	Ball kamani, partially ripe	0	0
7	Lemon, ripe	1	0
	Mango, ripe	4	58
	Ball kamani, ripe	3	114
8	Lemon, partially ripe	0	0
	Mango, partially ripe	8	54
	Ball kamani, partially ripe	4	376
9	Mango, nearly ripe	3	15
	Ball kamani, ripe but sound	0	0
	Lemon, beginning to turn color	0	0
10	Orange, ripe	1	10
	Mango, partially ripe	1	28
	Ball kamani, partially ripe	4	242
	Rose-apple (*Caryophyllus jambos*) nearly ripe	4	24
11	Orange, ripe	1	3
	Rose-apple, ripe	2	11
	Mango, ripe	11	126
12	Lemon, beginning to turn yellow	0	0
	Mango, partially ripe	3	15
	Ball kamani, mature but solid	0	0

HABITS OF MEDITERRANEAN FRUIT FLY

For those unfamiliar with the Mediterranean fruit fly it may be briefly stated that this pest belongs to the order Diptera and the family Trypetidae. It is one of many species of this family that cause much injury by their attack upon various fruits. In the Hawaiian Islands the fly attacks over 30 different species of fruits and has caused great financial loss. The adult female, which is about the size of the ordinary house fly, pierces the skin of the host fruit and forms an egg cavity beneath, in which she deposits eggs. The larvæ which hatch from these eggs either burrow at once to the center of the fruit, as in the peach (*Amygdalus persica*), or may feed in the outer portion, as in the star-apple (*Chrysophyllum*

cainito). In either case the fruit is rendered worthless by the developing larvæ before it is ripe. When the larvæ become well grown, they leave the fruit (Pl. XL) either before or after it has fallen and enter the ground or other protected places and transform to the pupal stage, from which the adult later emerges. In Hawaii the Mediterranean fruit fly requires in passing from the egg to the adult stage from 14½ days in summer to about 47 days during the coldest winter weather. In this paper the words "puncture" and "egg cavity" are often used synonymously.

PROPORTION OF EGG PUNCTURES CONTAINING EGGS

The data in Table I show that many of the punctures in the rind made by the female contain no eggs. In one of the most favored host fruits, the peach, practically all the punctures made contain eggs. Of 534 punctures made in 112 peaches but 13 were empty. The rind of lemon contains a much higher percentage of empty punctures than that of any of the other citrous fruits in Hawaii, except the Kusaie lime (*Citrus limetta*). In the 50 fruits examined 380 empty punctures were found, as compared with 185 with eggs. Practically all punctures in Chinese oranges contain eggs. (See Table I.) In the 85 fruits examined only 1 puncture out of 116 was empty. Grapefruit, or pomelos, shaddocks, and sour oranges seem to be preferred for oviposition to the ordinary budded or seedling oranges. It has been noted that adult fruit flies, especially the males, congregate in large numbers on citrous trees, and in the laboratory both sexes are quickly attracted to pieces of cut rind of citrous fruits. They seem to take pleasure in feeding upon the oils and other substances contained in the broken cells, and it is possible that in the field their liking for juices made available by the process of forming the egg cavity is so great that the females discontinue ovipositing and begin feeding. The large percentage of empty punctures in lemons and Kusaie limes, in particular, can not be ascribed to a lack of ripeness, as in practically all instances the fruits examined were fully grown and a large percentage were colored and overripe.

MORTALITY OF EGGS AND LARVÆ

Although many punctures in citrous fruits may be empty, others contain a sufficient number of eggs to infest badly a fruit not so well equipped by nature to withstand attack. Out of 13 punctures in one grapefruit 9 contained 76, 153, 32, 25, 18, 8, 46, 113, and 9 eggs, respectively. While this is a larger number of eggs than is usually found in a like number of punctures, it is sufficient when supplemented by the data from other citrous fruits to arouse interest in finding a reason why, with so many eggs deposited in citrous fruits, so very few flies succeed in reaching maturity. (See Table I.) Thirty-nine oranges, either yellow or orange in color, picked from the trees on September 13, 1913, and containing an average of 32 punctures, with a maximum of 108 and a minimum of 7

punctures, developed no flies, and their pulp was in a sound though somewhat shrunken condition after they had been held in the laboratory for one month.

That there takes place in citrous fruits a very great and previously unrecorded mortality among the eggs and larvæ is clearly set forth in the data in Table I. This mortality is especially pronounced in grapefruit, lemons, sweet oranges, and Kusaie limes in Hawaii, is less in Hawaiian limes and sour oranges, and very much less in Chinese oranges. It has been a common belief among many in Hawaii that citrous fruits are too acid to permit the larvæ to live in their pulp until ripe, in spite of the contradictory evidence that the quite acid Chinese orange is generally infested. The data in Table III are here given in proof that no citrous fruit, not even the lemon, is too acid for the development of Mediterranean fruit-fly larvæ. A study of the data shows that there is a high mortality among larvæ transferred to citrous fruits. Too much importance, however, should not be placed upon this, as these fruits must be mutilated somewhat in the process of transferring the larvæ and therefore are more easily attacked by decay fungi, which bring about a condition not especially desirable for the growth of larvæ and often positively fatal to their development. The data are of special interest in proving that even first-instar larvæ are able to reach maturity in well-grown though green lemons. The percentage of first-instar larvæ maturing in green lemons was in several instances even greater than that of larvæ maturing in ripe lemons.

TABLE III.—*Development of larvæ of Mediterranean fruit fly in citrous fruits*

Transference of larvæ.			Instar.	Number of larvæ.		
From—	To—	Date.		Transferred.	Died.	Matured.
Ball kamani	Ripe lemon	Feb. 19	First	41	23	18
Winged kamani (*Terminalia catappa*).	do	12	do	120	120	0
Chinese orange	Green lemon	28	do	17	17	0
Apple	California lemon	Mar. 12 to 15	do	480	381	99
Ball kamani	Ripe lemon	Feb. 20	Second	160	117	43
Do	Green lemon	20	do	20	17	3
Winged kamani	Ripe lemon	12 to 17	do	120	110	10
Chinese orange	do	14 to 18	do	40	32	8
Do	Green lemon	18 and 19	do	40	26	14
Ball kamani	Ripe lemon	18	Third	60	33	27
Winged kamani	Green lemon	16	do	80	50	30
Do	Ripe lemon	12 to 16	do	260	207	53
Chinese orange	Green lemon	18	do	60	48	12
Do	Ripe lemon	18	do	220	125	95
Ball kamani	California grapefruit	20 and 21	First	80	71	9
Winged kamani	do	21	do	20	13	7
Do	do	13 to 21	Second	120	67	53
Ball kamani	do	20 to 22	do	240	137	103
Do	do	20 and 21	Third	60	27	33
Winged kamani	do	21	do	60	22	38
Papaya	Ripe sweet orange	16	First	20	12	8
Chinese orange	do	20	do	20	20	0
Winged kamani	do	12	do	40	16	24
Do	do	12 and 13	Second	140	65	75
Papaya (*Carica papaya*)	do	17	do	40	28	12
Chinese orange	do	16	do	60	32	28
Winged kamani	do	13 to 16	Third	120	24	96
Papaya	do	17	do	40	20	20
Chinese orange	do	16	do	90	39	51
Ball kamani	do	12	do	40	8	32

The data in the tables make it evident that the cause of the mortality is not the acidity of the fruit. The figures are of interest in showing that the mortality occurs largely in the rind, either among the eggs in the punctures or among the newly hatched larvæ in the egg cavity where they hatch or in the rag beneath. The percentages of mortality occurring among eggs and newly hatched larvæ are given in Table IV.

TABLE IV.—*Mortality of eggs and larvæ of Mediterranean fruit fly in the rind*

Kind of fruit.	Total number of forms examined.	Mortality in percentages in rind.		
		Among eggs.	Among larvæ.	Total.
Grapefruit	8,222	90.5	9.3	99.8
Lemons	991	76.1	21.0	97.1
Limes	1,054	43.1	55.6	98.7
Kusaie limes	838	47.6	51.8	99.4
Sweet oranges	3,635	19.0	79.0	98.0
Sour oranges	2,357	71.7	17.3	89.0
Shaddock No. 1	1,155	3.8	95.3	99.1
Shaddock No. 2	303	13.5	18.1	31.6
Chinese orange	1,039	3.7	46.5	51.2

MORTALITY AMONG EGGS

As eggs deposited in such host fruits as the peach and loquat hatch with great certainty, the writers were of the opinion that the oil in the oil cells of the rind was an active agent in killing the eggs in citrous fruits. In puncturing the rind in the process of forming the egg cavity the female is likely to drill through one or several oil cells and the oil thus freed, though not of sufficient quantity to drive the female away, is sufficient in many instances to kill all or many of the eggs deposited. The data in Table V indicate that there is no question that the oil causes the death of the eggs. Only 163 out of 1,600 eggs treated with oil hatched, as compared with 1,313 out of 1,600 eggs held as a check. The eggs under observation were dissected out of punctures in California apples and placed on fresh foliage in moist jars. The treated eggs were not sprayed according to the usual method, but by bending over them a portion of the rind of fresh orange (in the first record) or fresh lemon (in the second and third records) so that the oil from the ruptured cells reached the eggs in that fine mistlike spray familiar to all who have eaten freshly gathered oranges. The much larger number of treated eggs that hatched in the last record is accounted for by the writers by their being fully 20 hours older than those in the second lot when treated. It should be stated that eggs removed from their host do not usually all hatch, as some sustain slight injuries and others may be infertile. See Table V.

TABLE V.—*Effect of oil from rind of orange and lemon upon the hatching of eggs of the Mediterranean fruit fly*

Period of depositing eggs.	Treated with oil.	Check.	Number of eggs hatched.	
			Treated.	Check.
1.30 p. m., Mar. 22, to 9 a. m., Mar. 23	800	800	41	609
9 a. m. to 1 p. m., Mar. 27	400	400	3	342
9 a. m., Mar. 27, to 9 a. m., Mar. 28	400	400	119	364
Total	1,600	1,600	163	1,313

Further evidence that the oil in the ruptured cells is the killing agent is the very small mortality among the eggs deposited in the Chinese oranges. Since in this fruit the rind is only about two twenty-fifths of an inch in thickness, the female is compelled to deposit her eggs either through the rind into the pulp or in a position between and parallel to the rind and pulp, but at a distance from the puncture that seems to be a protection from any oil set free by the puncturing process. Of 609 eggs thus deposited between the rind and the pulp 600, or 98.5 per cent, hatched, as determined by an examination of 85 fruits one week after they had been picked. A comparison of the 98.5 per cent hatched in Chinese oranges with the percentage of the mortality among eggs in other citrous fruits emphasizes the part the oil has in causing mortality among eggs. It is also interesting to note in passing that the eggs in Kusaie limes, the rind of which is sufficiently thick so that the eggs are deposited directly beneath the puncture, die with great regularity, while the eggs in Hawaiian limes, the rind of which may be sufficiently thin to permit the eggs being deposited as in Chinese oranges or so thick (according to the individual tree) that the eggs are laid either in the cavity in the rind or between the rind and pulp but directly beneath the puncture, suffer a degree of mortality between that of eggs deposited in Chinese oranges and Kusaie limes.

While in Chinese oranges the eggs deposited between and parallel to the rind and pulp hatch with great regularity, those deposited through the rind into the pulp are subjected to a mortality caused either by excessive moisture or lack of air. Eggs thus laid are usually placed beneath the skin covering the pulp, and the fascicle which they compose appears, after the rind has been removed, as a dull white spot that is easily overlooked. Usually no trace of the opening through the skin covering the pulp through which the eggs have been deposited can be found. The eggs appear thoroughly sealed within the pulp. In some few instances the opening is distinct and occasionally an egg is left in it, half in and half out of the pulp. When these openings in the skin occur, the eggs appear to hatch normally. Egg masses deposited entirely within the pulp may be located externally a few days after oviposition

by a round white sunken area in the rind which varies in size with the passing of time up to an inch in diameter. Of 560 eggs found in the examination of the above-mentioned 85 Chinese oranges 471, or 84.1 per cent, were unhatched and dead. This same kind of mortality occurs to a less extent in ordinary Hawaiian limes, but with no regularity, as the rind of these fruits in most instances is so thick that the female can not place her eggs within the pulp.

MORTALITY AMONG LARVÆ

It has been shown that mortality among the eggs occurs in the rind and in the pulp. Larval mortality occurs chiefly during the first instar, either in the egg cavity or in the rag beneath. Though mortality does occur in the pulp to a slight degree, no further notice of it will be taken, as it has little bearing upon the general purpose of this paper. The data in Table I show that of 6,571 larvæ recorded as dead but 18 died in the pulp, while 3,166 died in the egg cavities where they hatched, and 3,387 in the rag of the rind. The causes for this mortality of larvæ in the rind are threefold: The oil from the ruptured cells, the texture of the walls of the puncture, and the texture of the rag.

In the treatment of the eggs with oil, as recorded in Table V, it was found that of the larvæ hatching from the 163 eggs out of the lot of 1,600 eggs sprayed all died either before they were entirely out of the eggshell or before they had crawled much more than one-fourth of an inch. They exhibited a general weakness entirely lacking in normal larvæ. Larvæ hatching from check eggs were normal and crawled actively to all parts of the containing vials. As the oil sprayed on the eggs and foliage on which the eggs rested appeared to have entirely evaporated by the time of hatching, the writers believe that the few larvæ that succeeded in emerging from the eggs died from weakness imparted to the developing embryo by the oil with which the eggs were sprayed rather than from the effect of any oil still on the foliage with which they came in contact on hatching. Subsequent experiments have shown this supposition to have been correct. The writers believe, therefore, that the very large percentage of the deaths among newly hatched larvæ occurring in the egg cavity is the result of the action of the oil liberated during the formation of the cavity—oil which is sufficiently abundant to weaken the developing embryo but not abundant enough to kill the egg.

To such weakened larvæ and probably to many other normal larvæ hatching in egg cavities made without the rupture of oil cells the texture of the walls of the cavity present another difficulty. In many host fruits, such as the peach and loquat, the eggs are crowded into and completely fill the cavity made by the female, but shortly after oviposition, possibly as the result of the action of a fluid introduced by the female with the eggs, the flesh of the host shrinks considerably from the eggs, thus usually

leaving the eggs well separated and with ample room, making conditions favorable for the newly hatched larvæ. (See figs. 1 and 2.) On the other hand, in citrous fruits no such enlargement of the egg cavity takes place. Instead there occurs a general hardening of the walls of the cavity, and the eggs remain as tightly packed as when deposited. In many instances the cavity walls become almost woody. In fact the egg cavities in all the thicker skinned citrous fruits, such as grapefruit, lemons, and sweet oranges, resemble a gall the cavity of which is filled with eggs and the opening more or less clogged with a yellowish substance from the ruptured cells, and in Hawaii still further sealed by exudations of gummy secretions, especially in certain grapefruit, limes, and lemons. These gall-like cavities in the rind do not share in the general withering of the rind that takes place in citrous fruits after they have been picked for some days, but stand out from the general surface as small nodosities. (See fig. 3.) It is usual in host fruits of the fruit fly for the punctured surface to develop a depression. These thickened and often woody walls of the cavity no doubt offer an obstacle to the larvæ reaching the rest of the fruit which they can not overcome; hence, the larvæ are forced either to die in the cavity itself or to work their way out through the opening of the puncture to the surface of the fruit. It is probably seldom that larvæ leave the fruit by way of the opening of the puncture, but a few newly hatched larvæ have been found by the writers with their bodies half way out of the fruit.

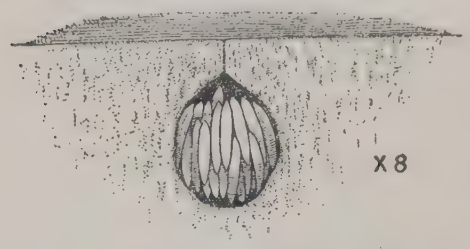

FIG. 1.—Cross section of peach, showing egg cavity of the Mediterranean fruit fly with eggs. Drawing made directly after oviposition. Original.

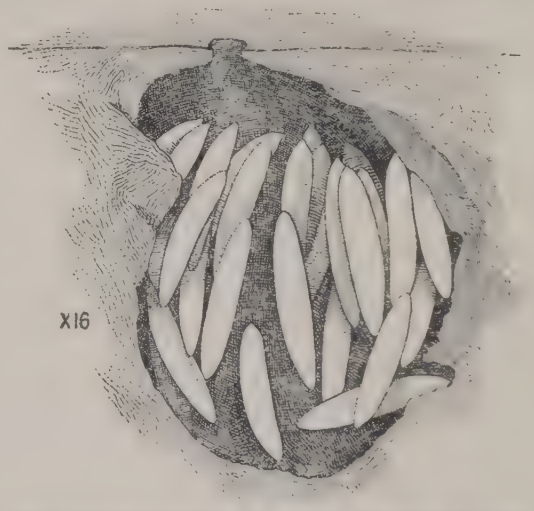

FIG. 2.—Cross section of peach, showing the general shriveling of the walls of the egg cavity and the separation of the eggs. Drawing made 1½ days after oviposition. Original.

Larvæ that succeed in getting out of the cavity must burrow through the rag before reaching the pulp, and this is a difficult task, as evidenced by the fact that out of 3,345 newly hatched larvæ that succeeded in reaching the rag, as shown in Table I,[1] 3,276, or 97.9 per cent, died in the rag. The larvæ, after leaving the egg cavity, burrow in all directions, but seldom get more than 1 inch from the cavity and usually not that far. Often they are able to reach the skin covering the pulp or to burrow

[1] This number excludes shaddock No. 1 and sour and Chinese oranges, which are of no commercial value and are more easily infested than grapefruit, lemons, limes, and sweet oranges.

down between the sections of the fruit, but seem to be lacking in strength to penetrate the skin after they have reached it. There apparently is nothing in the rag itself as a food to cause the death of the larvæ, as larvæ can attain full growth when feeding on the rag of certain shaddocks. Whether larvæ die or not seems dependent upon the degree of toughness of the rag, and the closeness with which the rag adheres to the skin covering the pulp. The toughest rag found was that of sweet oranges still hanging on the tree in March in a much overripe condition. These fruits had begun to be pithy at the stem end, and the rind, which was more or less russeted, had begun to wither and yielded no oil when sharply bent. These fruits were very much like the overripe, badly russeted seedling oranges frequently found on trees in Florida during April and May as "leftovers" from the winter crop. In the 20 fruits examined, containing an average of 7 punctures to the fruit, no larva was able to penetrate the rag. The coarsest rag or that with the loosest texture is that found in certain large shaddocks growing in Hilo, Hawaii (Pl. XLI). These fruits were much overripe when gathered from the tree in March. An examination of 14 of these fruits showed that out of 245 larvæ, mostly in the third instar, present in the rag and pulp, 152 were alive in the rag, 55 alive in the pulp, and only 38 dead, all in the rag. Fourteen other shaddocks, apparently in the same state of ripeness but growing on another tree and so very much undersized as to resemble a medium-sized grapefruit, had a very much tougher rag. An examination of these fruits showed that but 5 very young larvæ out of 701 found in the rag were alive and that no larvæ had succeeded in penetrating the pulp. The data in Table I show that in the grapefruit, lemons, limes, and sweet oranges examined, 326, 15, 424, and 1,552 first-instar larvæ, respectively, died in their attempt to puncture the rag, as compared with but 1 first-instar larva found alive in the rag of grapefruit and 17 third-instar larvæ found in the pulp of sweet oranges.

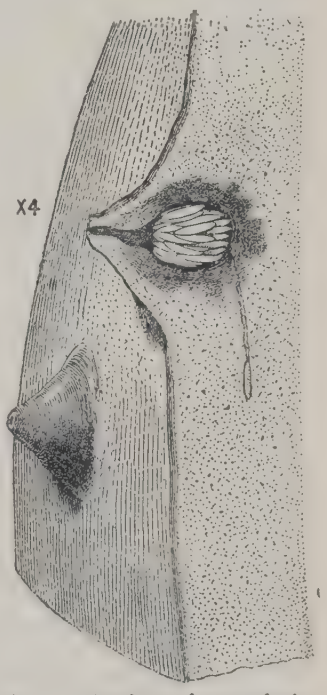

FIG. 3.—Section of grapefruit rind, showing two egg cavities, one in cross section. Drawing made one week after fruit was picked. Note conical elevation about the egg cavities left by the withering of the rind; also the thickened walls of the egg cavity and the single larval channel in the rag. Original.

The ordinary sour orange of Hawaii, which is identical with that grown in Florida, possesses a loosely attached rind the rag of which is much looser in texture and from the standpoint of imperviousness to the young larvæ seems half way between that of the ordinary sweet orange and large well-ri[illegible]ened shaddocks. After the larvæ have succeeded in passing through the rag of these oranges they work their way between the rag and the skin and finally enter the pulp, usually at the blossom end.

Hawaiian limes possess less rag than sour oranges and the larvæ reach the pulp more easily. In 1,692 ripe and yellow limes picked during April, showing an average of about 5 punctures to the fruit, the larvæ succeeded in reaching the pulp in but 287 cases. Chinese oranges have been shown to be generally infested because they possess a very thin, loosely fitting rind, and for practical purposes may be said to possess no rag. Unfortunately the writers have had but little experience with tangerines (*Citrus nobilis*), as these are rarely found in Hawaii, but such few fruits as have come to their attention have been well infested, which is to be expected, because of their thin, loosely fitting rind and rag.

PERSISTENT ATTACK LEADING TO INFESTATION OF THE PULP

Laboratory experiments and field examinations have shown that the female fly seldom deposits more than six eggs in a puncture at one time. So well has nature equipped the average citrous fruit to withstand attack that it is doubtful whether such fruits as the grapefruit, lemon, or orange would ever become infested [1] until very much overripe, if the female fly formed a new puncture for each batch of eggs deposited, thus making it necessary for the larvæ hatching from each lot of eggs to face identical difficulties in reaching the pulp. This, however, she does not always do. As many as 153 eggs have been taken from a single puncture in grapefruit. A very large number of punctures contained more eggs than the female deposits normally at one oviposition. It is very evident, therefore, that females oviposit in a large number of instances in the same puncture rather than make a fresh puncture for each batch of eggs. Frequently freshly laid eggs have been found in egg cavities from which channels made by larvæ from previously deposited batches of eggs extend through, to, and into the pulp or in punctured areas of the rind showing dry decay which is known from observation to have been forming for fully one month. Usually the rag beneath a puncture develops a discolored area, no matter whether the puncture originally contained eggs or not, and very often this discoloration of the rag, which appears to be caused by a dry rot, extends to the outer rind and causes deadened, sunken areas to form about the punctures. Such blackened areas, which had been developing in the rind of well-punctured oranges held at the laboratory for one month after picking, are shown in Plate XLII, figure 2.

It has already been stated that many larvæ die in the rag and that before dying some of these larvæ channel through the rag in all directions. Often all the larvæ escaping from a puncture will be found dead next the skin protecting the pulp; again they will be found dead at the heads of channels extending fully 1 inch from the puncture. In large shaddocks they may even channel 3 or 4 inches through the loose rag (Pl. XLI).

[1] The term "infested" is here applied to fruits which have larvæ in the pulp, show decay, and become generally unfit for consumption.

It is evident, therefore, that larvæ hatching from the successive batches of eggs deposited in punctures or in the decayed areas of the rind forming about the punctures find conditions increasingly favorable to their ultimate success in reaching the pulp. The longer the fruit is allowed to remain on the tree after it becomes ripe, the easier it is for the maggots to reach the pulp. The rind can not withstand indefinitely the persistent attack of successive lots of larvæ and the work of decay fungi to which the punctures give entry (Pl. XLII, fig. 1). Thus, 39 sweet oranges showing an average of 32 punctures to the fruit, gathered from the trees in September, 1913, at a time when they were just becoming ripe, developed no larvæ. On the other hand, out of 784 sweet oranges gathered during March, 1914, in a very much overripe condition, 254 produced 2,272 larvæ, or an average of about 9 larvæ to the fruit. On account of the looseness of the rind and rag of sour oranges and the greater ease with which the rind is destroyed by decay fungi, these fruits are more quickly infested by the fruit-fly larvæ.

While both sweet and sour oranges in an overripe condition ultimately succumb to the repeated attacks of the Mediterranean fruit fly if permitted to remain on the tree, lemons, both of the commercial smooth-skinned and the rough-skinned varieties, withstand these attacks with a constancy that is astonishing. Lemons are not grown in sufficiently large numbers in Hawaii to permit the writers to record observations on large quantities of fruit, but even in orchards where the fruit is heavily punctured infested fruits are very seldom found. In about two years' time only three infested lemons of the commercial variety and one of the rough-skinned variety have been seen by the writers or by fruit-fly inspectors. Out of 235 well-grown and for the most part ripe lemons of the commercial type, picked from the tree, only 1 developed larvæ (this contained 3), and this fruit when picked was partially decayed as a result of a thorn prick. Out of 161 lemons of the same variety, taken from the ground in a very much overripe condition, but 2 developed larvæ—1 and 5, respectively. No larvæ developed in 434 ripe rough-skinned but badly punctured lemons picked from the tree. One partially decayed rough-skinned lemon taken from the ground produced 12 larvæ.

The thicker skinned grapefruit, such as the writers have had an opportunity to study best, have shown a strong resistance to the repeated attack of larvæ or fungi. Yet these fruits were all grown on less than a dozen trees and in one garden. Twenty-five fruits taken from beneath these trees in a very ripe condition and showing an infestation of the rind equaling that recorded in Table I of the 39 fruits picked from the same trees, produced no larvæ in the pulp. However, larvæ have been found in a few thin-skinned grapefruit that were in a very much overripe condition.

SECONDARY ATTACK OF CITROUS FRUITS BY INSECTS OTHER THAN THE FRUIT FLY AND BY FUNGI

The excellent experimental work of the Bureau of Plant Industry carried on in Florida during the last few years has forcibly demonstrated the causes of decay of citrous fruits in transit from orchard to market. Mechanical injuries to the rind have been found to be a fertile source of trouble by furnishing entry for decay fungi. The writers believe that a great share of the decay of oranges en route to market, recorded in the early history of the Mediterranean fruit fly, was caused more by insanitary conditions in the holds of ships than directly by fruit-fly larvæ. It is more than likely that the oranges shipped from the Madeira Islands and the Azores to London contained fruit-fly punctures which greatly aided the blue mold in its destructive work.

Statements made by the early writers and even repeated in the Hawaiian Islands at the present time, that citrous fruits drop as soon as punctured, are untrue. There is no such thing as a general shedding of fruits following puncturing of the rind. Oranges and grapefruit have been known by the writers to hang on the tree from two to three months after they were first punctured. It is probable that the wholesale shedding of fruit recorded by others was caused by fungi or physiological troubles.

Species of Drosophila and Bruchus may usually be found ovipositing in breaks in the rind of Citrus made by the Mediterranean fruit fly. Their persistent attack, supplemented by decay fungi, causes an appreciable amount of decay in Hawaii.

EFFECT OF ATTACK OF THE MEDITERRANEAN FRUIT FLY UPON CITROUS CROPS OF CALIFORNIA AND FLORIDA

In the opening paragraph the writers made the statement that their investigations in Hawaii have led them to believe that even if the Mediterranean fruit fly should be introduced into the citrous regions of the United States it would not become a serious pest to citrous fruits. In the Hawaiian Islands, especially in the lowlands, climatic conditions are more favorable for the rapid increase of the fruit fly than they are in any section of the United States or of the Mediterranean regions where oranges are grown commercially. The monthly mean temperatures at Honolulu during 1912 and 1913 ranged from 69.6° to 79.2° F. During the hottest summer weather the fruit fly requires a minimum of about 14½ days to complete its life cycle from egg to adult. During late December, 1913, and January and early February, 1914, it required many flies fully 47 days to reach maturity in common guavas (*Psidium guajava*). During March and April, 1914, the fruit fly required from 20 to 30 days to pass from egg to adult in half-ripe peaches and from 28 to 40 days in lemons. In Bermuda during December and January,

when the monthly mean temperature normally ranges from 62.5° to 64.8° F., the senior writer, with the kind assistance of Mr. E. J. Wortley, Director of Agriculture, Bermuda Agricultural Station, found that the length of the pupal stage was about 31 days, which would make the period for development from egg to adult about 58 days in favored hosts. In Honolulu the cold-storage experiments of the writers have shown that the fruit fly requires about 91 days to complete the same development at about 56° F. A temperature of 54° to 57° will not prevent adults from emerging from pupæ in cold storage, although it lengthens the pupal stage from 8 days, a normal minimum required at Honolulu in warm weather, to 36 days. Very few eggs out of several hundred were able to hatch at a temperature of about 53° to 54°, while practically no eggs will hatch nor larvæ mature at a temperature of 50° F. A continued temperature ranging from 33° to 46° F. will kill pupæ and larvæ, although both may be subjected to these temperatures for short periods without apparent injury. Freezing temperatures have proved generally fatal to both larvæ and pupæ. At 45° larvæ are not able to pupate, although some hardy specimens may become active and pupate if removed at the end of a month from this temperature to the normal Honolulu summer temperature. A total of 10,203 second and third instar larvæ kept at a temperature varying from 42° to 46° were all dead at the end of 45 days, except one third-instar larva which was probably moribund, while out of 10,959 second and third instar larvæ kept at a temperature varying from 33° to 38° none were alive after the seventeenth day.

These data from the notes on file are given here to show that even the cool winter climate of the lowlands of Hawaii has a decided effect in checking the increase of the fruit fly, that temperatures as low as 56° F. greatly lengthen the life cycle, and that a temperature of 50° to 52° practically prevents eggs from hatching. Unfortunately no data are at hand on the effect of the temperature varying above and below a mean temperature ranging from 50° to 53°. Certain deductions, however, can be made from known facts regarding the development of the fruit fly in the Mediterranean region, especially in southern Spain, France, Italy, and Sicily, that show that the fly does not multiply, or at least undergoes an extremely slow development, when the monthly mean temperatures range from 50° to 54°. During the spring and summer of 1913, Prof. H. J. Quayle, of the University of California, investigated the status of the fruit fly in the Mediterranean regions for the Bureau of Entomology,[1] and his observations bore out the contentions of the present Italian entomologists that the fruit fly is not a serious pest to Citrus in Spain and Italy. The fact that Prof. Quayle found no evidence

[1] Quayle, H. J. Citrus fruit insects in Mediterranean countries. U. S. Dept. Agr., Bul. 134, 33 p., 2 fig., 10 pl. 1914.

of fruit-fly infestation in oranges and lemons in Spain during March or in southern Italy and in Sicily during April, May, and early June is strong evidence that the fruit fly is prevented by the mean temperatures prevailing in these countries from becoming a pest during the winter and spring months. Loquats are a preferred host of the fruit fly, being badly attacked when flies are present, and the appearance of infested fruits is such that infestation is easily detected. Even during July at Valencia, Spain, Prof. Quayle found but a slight infestation of peaches and overripe oranges. In August, near Palermo, Italy, peaches were found badly infested, but lemons growing in the midst of peach trees were not infested. Reports indicate that in Spain and southern Italy the fruit fly may cause some damage to ripening oranges during September and October, although this is slight and of short duration. Citrous fruits, especially oranges, are not usually punctured by the female fruit fly until they are well grown and about to turn color, and the period of time is short after they reach this stage of ripeness until cool weather renders the fly sluggish. The season of the year, therefore, when the bulk of the citrous crops are best suited for fruit-fly attack coincides with the season of inactivity of the fly due to lower temperatures.

The mean monthly temperatures given in Table VI indicate that the Mediterranean fruit fly would find conditions in the citrous regions of Florida and California quite similar to those in Spain and Sicily. The Florida temperature, especially in the citrous regions of southern Florida, is decidedly above the winter means of those of California. However, even if the temperatures were higher than they are, the writers feel that the fruit fly would assume a minor position as a citrous pest. The cool winter weather would have the same retarding effect upon development in California and Florida that it has in the European countries.

TABLE VI.—*Monthly mean temperatures in citrous regions*

Locality.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.
Seville, Spain	52.2	55.9	59.5	63.9	69.6	78.1	84.7	84.9	78.1	68.4	60.1	52.9
Malaga, Spain	53.6	55.4	57.6	61.5	65.7	71.4	76.8	77.2	72.5	66.0	60.3	55.4
Naples, Italy	46.8	48.4	51.4	56.8	63.7	70.3	75.6	75.0	69.8	63.1	54.7	48.7
Palermo, Sicily	50.5	52.1	54.7	56.8	64.0	70.7	76.3	76.6	73.4	67.3	59.4	53.4
San Francisco, Cal.	50.0	52.0	54.0	55.0	57.0	59.0	59.0	59.0	61.0	60.0	56.0	51.0
Redlands, Cal.	51.0	52.0	55.0	61.0	66.0	74.0	78.0	78.0	72.0	65.0	59.0	53.0
San Diego, Cal.	54.0	55.0	56.0	60.0	62.0	65.0	68.0	70.0	66.0	64.0	59.0	56.0
Portersville, Cal.	49.8	51.6	59.0	.....	.....	.....	.....	.....	.....	60.0	57.0	48.0
Jacksonville, Fla.	55.0	58.0	63.0	68.0	75.0	80.0	82.0	82.0	78.0	71.0	62.0	56.0
Eustis, Fla.	58.0	61.0	67.0	70.0	77.0	81.0	83.0	83.0	80.0	73.0	66.0	60.0
Miami, Fla.	65.0	67.0	71.0	74.0	76.0	81.0	82.0	82.0	81.0	78.0	74.0	69.0
Myers, Fla.	62.0	65.0	68.0	72.0	77.0	80.0	81.0	81.0	80.0	75.0	70.0	64.0
Honolulu, Hawaiian Islands	71.4	70.8	69.6	72.6	74.6	76.0	77.4	78.2	78.2	77.6	74.6	74.0
Prospect Hill, Bermuda	62.5	62.2	63.9	66.1	71.4	77.7	79.8	81.0	78.0	73.7	68.6	64.8

The general effect of retarded development of the fruit fly due to cold weather is to increase the mortality among all stages. Even pupæ are subject to an increasing rate of mortality the longer they are subjected to lower temperatures. Adults seem more able to withstand prolonged cold weather than any of the other stages. One individual was kept alive by daily feeding for 4½ months during a Hawaiian winter and spring. However, during the cooler months the adults are more sluggish and fall more easily a prey to adverse climatic conditions, such as heavy winds and rains, and to predaceous insects. Mr. George Compere reports having seen adults sunning themselves on orange trees in Spain after a night during which the temperature dropped to freezing, thus showing that adults can withstand temporarily any cold snap likely to occur in a citrous section. However, the fact that adults do not succeed in thriving during the winter temperatures of southern Spain and Italy and in Sicily seems to be well proved by the fact that it is only during the summer and early fall that the fruit fly becomes a serious pest in favored host fruits and in overripe citrous fruits. If this were not so, fruits would become badly infested much earlier in the season than they do. The number of adults surviving the winter must be very small. Even the mild winters of Hawaii at Honolulu have a very noticeable effect upon the numerical abundance of the adult flies, as shown by trap experiments extending over one full year.

In addition to this beneficial effect of lower winter temperatures, both California and Florida growers will receive further protection as a result of the conditions surrounding the growing of Citrus as a commercial proposition. In the Hawaiian Islands, especially about Honolulu, citrous fruits are subjected to the most severe attack imaginable under field conditions. They are attacked over long periods by an abundance of fruit flies that mature in many host fruits ripening at intervals throughout the year on all sides of isolated citrous trees. The number of wild fruits in which the fruit fly can breed in the citrous regions of California and Florida is, in comparison with Hawaii, so extremely small that the fly would find conditions unfavorable for rapid increase, even if weather conditions were more favorable. In many instances large acreages of Citrus occur where vegetation is normally decidedly stunted unless irrigation is practiced. With the excellent work of the horticultural inspectors in California a reduction of the noncitrous host fruits in and about citrous groves is a practical proposition. Even near-by orchards of drupe fruits are not the menace that they seem to many, inasmuch as their crops are unsuitable for fly attack except during short periods of the year. The very scarcity of vegetation that can not be destroyed which produces fruits subject to fruit-fly attack makes it possible to attach a far greater importance in California and Florida than in the Hawaiian Islands to the excessive mortality of the fly discussed in this paper. It has been shown

that it takes repeated attacks to infest grapefruit, lemons, and oranges in Hawaii and that the pulp of these fruits is infested usually only after the fruits are very ripe; in fact, not until they become much riper than commercially-grown oranges usually are allowed to become in either California or Florida, unless exception be made of such varieties as late Valencias. The relatively small number of adult fruit flies entering a block of citrous trees would find it very hard to establish themselves, since the numbers would be so insignificant as compared to the fruit surface suitable for oviposition that each female would be less likely to oviposit repeatedly in the same puncture. Her progeny would therefore meet with almost insurmountable difficulties in reaching the pulp.

It has been stated that in Hawaii citrous fruits offer themselves for attack over several months and that they are not subject to serious attack until they have turned or are about to turn color. It is a well-known fact among horticulturists that in very equable climates the pulp of oranges may be ripe enough to eat while the rind is still very green. In Florida and California this is not so true. One has only to visit the packing houses in either State to be convinced that much fruit is gathered for the early trade in a semiripe condition, or at least when the rind is quite green in color. The writers feel safe in saying that market conditions are such that early fruit is placed on the market at the earliest possible moment, in order that high prices may be secured. The fear of unseasonable frost and freezes has made it difficult for those who have the interests of the citrous industry at heart to prevert the shipping of too green fruit. It would seem that with a reasonable expenditure of more care than labor, citrous groves in either Florida or California can be made so well protected from the Mediterranean fruit-fly attack that such few flies as enter them during the fall will find the early fruit, upon which they can work because of its degree of ripeness, picked before they are able to injure it to any extent. The cold weather will protect the later fruit by rendering the fruit flies inactive, and by the time the spring temperatures become suitable for fly activity the bulk of the fruit will have been marketed and the numerical abundance of the adult flies greatly lessened.

In addition, if it becomes necessary, as a result of unfavorable conditions, to use artificial means of control, spraying with a cheap poisoned bait will be a practical method of reducing the number of adults. If the writers under most adverse conditions can reduce by spraying the number of adult fruit flies over 50 per cent in one city block in Honolulu, into which it has been proved that adults are continually migrating, it is only reasonable to expect that the same good results as have been secured in South Africa, where fruit has been protected by spraying, will follow spraying in either Florida or California, where outside sources of infestation can be so easily controlled.

CONCLUSION

Citrous fruits are not the favored host fruits of the Mediterranean fruit fly (*Ceratitis capitata* Wied.) that the earlier writers thought. While grapefruit, oranges, lemons, and many limes may become quite badly infested with well-grown larvæ if allowed to remain on the tree long after they become sufficiently ripe for the market, nature has so well equipped them to withstand attack that larvæ are seldom found in their pulp until they are much overripe. Oranges and grapefruit are generally eaten and found uninfested if gathered as they ripen. Indeed, in Honolulu, where conditions are very favorable to early infestation of the pulp, owing to the excessive numbers of adult flies breeding in a large number of host fruits ripening in rapid succession, it is doubtful whether grapefruit, oranges, and lemons would ever become infested until long after becoming overripe if the female fly formed a fresh egg cavity for each batch of eggs deposited, for the reason that the eggs and the young larvæ found in the egg cavity and in the rag of the rind would then be forced always to face well-nigh insurmountable difficulties. The oil of the cells ruptured in the formation of the egg cavities kills a large percentage of the eggs and newly-hatched larvæ. Larvæ that succeed in entering the rag from the egg cavity are able to reach the pulp in astonishingly small numbers because of the imperviousness of the rag. It is only the persistent attack of successive lots of larvæ hatching from different batches of eggs laid in the same puncture in which the oil has become inoperative that finally breaks down the barrier between the young larvæ and the pulp.

The Mediterranean fruit fly is quickly affected by low temperatures. A temperature of about 56° F. has lengthened the time required by the fly to pass from the egg to the adult stage from 14½ to 91 days. A temperature ranging from 50° to 55° F. will either seriously check development or kill large numbers of the immature stages of the fly. The winter monthly mean temperatures of California and Florida are so similar to those of the citrous regions of southern Spain and Italy and of Sicily that it is to be expected that the fruit fly, if introduced to the mainland, would not become a serious pest to *Citrus* spp. It happens that the very cold temperature necessary to bring citrous crops to that degree of perfection in which they are most susceptible to fruit-fly attack likewise renders the fly so inactive or sluggish that it may be disregarded as a pest for that period of the year.

In addition to the assistance of adverse climatic conditions during that part of the year when they are most needed to protect citrous crops, the growers of California and Florida are still further protected—and most admirably so—from attack by the very scarcity of wild host fruits that can not be destroyed. It will be found a practicable undertaking to remove such a number of noncitrous host plants at present

growing about commercial citrous orchards that the succession of fruits in which the Mediterranean fruit fly can breed during the large portion of the year when citrous fruits are unavailable for attack because of their greenness will be reduced to a minimum, if not entirely done away with. It is under conditions such as can be secured in California and Florida that the excessive mortality occurring in the rind will become a valuable factor in preventing infestation or establishment of the pest, as each fruit will in reality become a trap for stray females. The scarcity of host fruits will also make spraying with poisoned baits a practical undertaking, should it become necessary to resort to artificial methods of control.

Adverse climatic conditions at a season when citrous fruits are most susceptible to attack, solid plantings of Citrus in commercial orchards, a scarcity of noncitrous host fruits, the ease with which the fly can be reduced by spraying with poisoned baits, and the general practices followed in harvesting fruits make it possible for the citrous growers of California and Florida to rest assured that the discovery of the Mediterranean fruit fly in either State will not bring about the ruination of the industry. Its presence will be a constant menace, but it can be successfully fought.

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PLATE XL

Fig. 1.—Orange infested with larvæ of the Mediterranean fruit fly (*Ceratitis capitata*). Note that the fruit looks sound, except about the irregular hole, through which a few well-grown larvæ have already left the fruit. Original.

Fig. 2.—Orange infested with larvæ of the Mediterranean fruit fly (*Ceratitis capitata*), showing two breathing holes of the larvæ in the decayed area. Original.

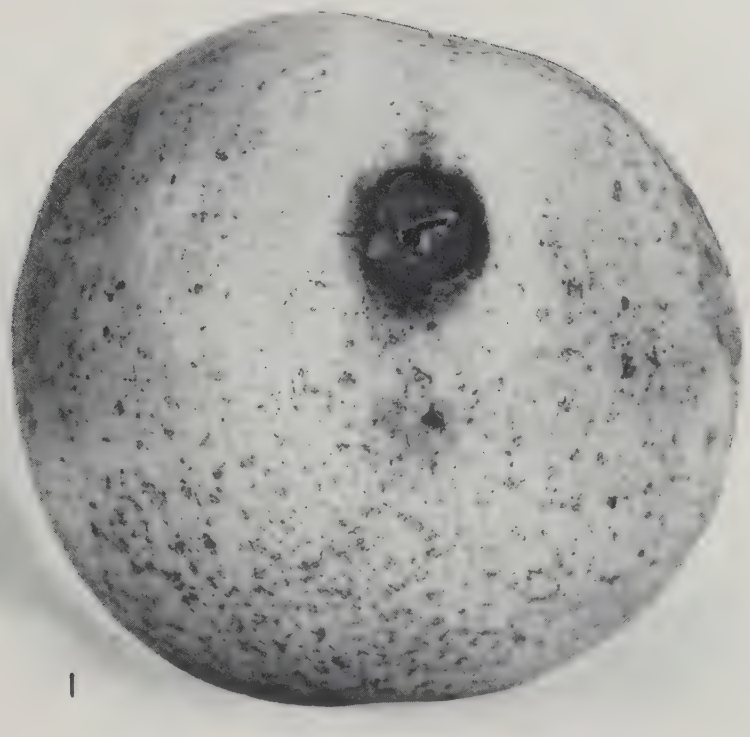

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PLATE XLI

Cross section of shaddock No. 1, showing the thick, loose texture of the rag with darkened area above and to the right showing the channels made by well-grown Mediterranean fruit-fly larvæ. Original.

PLATE XLII

Fig. 1.—Cross section of the orange shown on Plate XL, figure 2. Note that in this instance the larvæ have brought about decay in only one section. Often many sections are thus affected in very ripe fruits. Original.

Fig. 2.—Orange containing 87 punctures in the rind. Photographed one month after being picked from the tree in a ripe condition. Note that the rind about many punctures is sunken as a result of a dry black-rot. The pulp of this fruit was perfectly sound. Original.

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PHYSIOLOGICAL CHANGES IN SWEET POTATOES DURING STORAGE

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INTRODUCTION

In resting storage organs of plants growing in northern and in temperate regions, carbohydrate transformations involving the disappearance of reserve starch during the colder months and its temporary reappearance in spring have been found to be of general occurrence. The disappearance of starch from the cortex of trees in winter and its reappearance in early spring was first noted by Müller (1877),[1] who believed the absence of starch in the cortex resulted from its migration into the wood. Russow's investigations (1882–83), which included the examination of a number of tropical and subtropical greenhouse plants, showed that the total or partial disappearance of starch from the cortex of woody plants in winter was a phenomenon of widespread occurrence. He found, however, that the starch did not migrate into the wood, as Müller supposed, for when pieces of cortex chiseled from trunks of trees were kept at a temperature of 14° to 17° R. (17° to 21° C.) starch grains began to reappear in 20 hours. In the tissues which were free from starch in winter he found oil and fats. He observed a correlation between the temperature and the disappearance and reappearance of starch, but since the processes occurred also in tropical plants in the greenhouse, he did not regard temperature changes or climatic conditions as the prime causes of the observed transformations. Later Grebnitzky (1884) and Baranetzky (1884) showed that the starch of soft-wooded trees disappeared entirely from the wood, cortex, and rays in winter, and that oil appeared in its place, while in hardwood trees the starch disappeared from the cortex, but persisted in the wood. Fischer (1891), in his extended investigations on the physiology of woody plants, fully confirmed the observations of Russow (1882–83), Grebnitzky (1884), and Baranetzky (1884) regarding the appearance of oil in place of starch in soft-wooded trees, and showed further that in hardwood trees glucose and tannin are present in the cortex after the disappearance of the starch, and that the glucose, but not the tannin, disappears when starch is regenerated. He found that the regeneration of starch takes place at a temperature only a few degrees above 0° C. The minimum temperature at which he observed the regeneration of starch in twigs was about 5° C., while at 10° to 20° the process went on very rapidly.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 341.

That the periodic transformation of reserve starch is not restricted to the stem tissues of plants is shown by the observations of Haberlandt (1876), of Mer (1876), and of Schulz (1888), who found that the starch disappears from evergreen leaves in temperate regions in winter, while Haberlandt and Schulz noted also that it was re-formed in spring. The most thorough investigation of the carbohydrate transformations in evergreen leaves was made by Lidforss (1907), who found that the leaves of all evergreen plants in cold countries, except aquatic plants, lose their starch in winter, sugar appearing in its place, and that starch is regenerated in the leaves in February and March when the temperature scarcely rises above 5° C.

That similar changes occur in the subterranean parts of perennial plants in temperate regions was shown by Rosenberg (1896), who observed the disappearance of starch after leaf fall in the subterranean parts of *Spiraea ulmaria*, *Scrophularia nodosa*, *Plantago major*, *Potentilla argentea*, and *Hepatica triloba*, but did not determine what substances appeared in its place. By far the most complete account of carbohydrate transformations in dormant organs of this type is given by Müller-Thurgau (1882) in his classical researches on the accumulation of sugar in the potato (*Solanum tuberosum*) and other plant organs at low temperatures. Müller-Thurgau found that an accumulation of sugar and a corresponding loss of starch occurred in potatoes kept at low temperatures (0° to 6° C.), while, contrary to popular opinion, no sugar is formed in potatoes which have been actually frozen. He found that when potatoes which had become sweet as a result of exposure to low temperature are kept at a higher temperature (8° to 10° C.) the sugar disappears and the starch increases. Furthermore, he showed that the sugar formed consists mostly of reducing sugar with some cane sugar in the proportion of about 2.5 to 1, and that similar transformations occur in other parts of plants. These phenomena are interpreted by Müller-Thurgau (1882) as follows:

The transformation of starch into sugar is an enzymic process which, although more rapid at high temperatures, occurs also at low temperatures. The respiratory activity which is almost at a standstill at 0° C. rises with the temperature so that at higher temperatures an increasingly greater amount of sugar is consumed by respiration. The amount of sugar used in respiration at higher temperatures is, however, small compared with that utilized by another process—i. e., the re-formation of starch from sugar, which takes place at temperatures somewhat above 0° C. and increases in speed with the rise of temperature. Appleman (1914) in his studies on the rest period of the potato also finds that the carbohydrate changes in the dormant tubers are entirely dependent upon changes of temperature. It appears, therefore, that the carbohydrate transformations of the potato, although a

subtemperate plant and not capable of long withstanding temperatures much below freezing, resemble in their general trend those of subterranean organs of temperate plants. In the more strictly tropical sweet potato (*Ipomoea batatas*) carbohydrate transformations of a similar nature have been observed. Thus, Harrington (1895) found that in stored sweet potatoes there was an increase of the total amount of sugar up to March 6, beyond which the experiments were not continued. Shiver (1901), whose experiments were somewhat more extensive, found that during the time of his experiments (up to April 17) there was a gradual decrease of starch and an increase of cane sugar, while the invert sugar showed but slight fluctuations. Neither of these writers described the conditions under which the potatoes were stored nor attempted to determine the effect of temperature on the metabolic changes.

The storage of sweet potatoes is accompanied by considerable losses as a result of decay which is not wholly preventable by any of the methods of storage advocated at present. The decay is brought about by microorganisms which invade the tissues. In the matter of susceptibility the internal changes in the roots must play an important part. These changes are affected by changes in temperature and other conditions to which the roots are subjected during storage. It is therefore a matter of practical importance, as well as of theoretical interest, to study the internal changes which take place in sweet-potato roots after harvest and during storage, and to determine the effect of external conditions upon such changes. The work reported in this paper is a general study of the carbohydrate metabolism of sweet potatoes stored at different temperatures.

PLAN OF THE EXPERIMENTS

For the purpose of this work two varieties of sweet potatoes, the Jersey Big Stem, representing the sugary type, and the Southern Queen, representing the starchy type, were selected. The potatoes used in the experiments were a part of the general crop grown by the Office of Horticultural and Pomological Investigations during the summer of 1911 in a series of variety tests which had been continued for a number of years. At the time of harvesting, a representative lot of about 15 bushels of each of the two varieties was selected in the field and packed in slat crates holding about a bushel each. These were placed with the rest of the crop in the sweet-potato cellar of the Office of Horticultural and Pomological Investigations, where all were subjected to the "sweating," or curing, process. During the period of curing, the temperature of the room was kept at approximately 27° C. for about 10 days, after which it was allowed to drop to the regular storage temperature, ranging in this case, except near the end of the season, between 11.7° and 16.7° C. Nine crates of each variety were left in the cellar at the above-mentioned

temperature, which was maintained by the aid of artificial heat when necessary. The remaining six crates of each variety were placed in a cold-storage room, which was kept at a fairly uniform temperature of 4° C., by means of circulating brine cooled by ice and salt. Thus, both the lot stored at the usual storage temperature and that placed in cold storage were submitted to the same preparatory curing process. In order to determine the carbohydrate changes which occurred in these lots during the season, samples were analyzed on the day the potatoes were dug and at intervals of about a month during the course of the experiment, from October to June. In these samples the water, starch, reducing sugar, and total sugar were determined.

EXPERIMENTAL METHODS

SAMPLING.—For each set of determinations, a random sample of 4 to 5 kg. was taken. The roots were rapidly washed and wiped with a towel. When the surface had become entirely dry the roots were cut up as quickly as possible and ground in a power-driven meat grinder having a face plate with holes 3.2 mm. in diameter. The operation of cutting and grinding required about 10 minutes. The mash thus obtained was thoroughly mixed on a glass plate and quartered twice. The final sample thus obtained was placed in a crystallizing dish and covered with a damp towel while the samples for sugar, starch, and moisture determinations were being weighed out.

MOISTURE.—For the determination of moisture, samples of approximately 10 gm. were transferred into tared weighing bottles and accurately weighed. The material was covered with 95 per cent alcohol, which was subsequently evaporated in vacuum desiccators containing sulphuric acid. The samples were then dried to their lowest weight in a current of hydrogen in a vacuum oven at 78° C. The drying required 15 to 18 hours, during which the bottles were weighed three or four times.

STARCH.—It was not possible to make the starch determinations immediately. Samples of 25 gm. correctly weighed to 1 cm. were therefore transferred to Erlenmeyer flasks of 200 or 250 c. c. capacity and covered with 150 c. c. of 95 per cent alcohol. A little precipitated calcium carbonate was added to the flasks, which were then brought to the boiling point in a water bath. Subsequently the samples were washed with alcohol into tared porcelain extraction thimbles, 75 mm. high and 40 mm. in diameter, with perforated bottoms which were covered with filter paper cut to fit. Another piece of filter paper was pressed down upon the material and held in place by means of a cotton plug. The thimbles were supported well up in Soxhlet extraction apparatus and extracted with strong alcohol for 12 hours. After extraction the cotton and filter paper were removed, and the thimbles were

dried for 20 hours at 60° C. and subsequently were allowed to stand in the laboratory at least 48 hours, in order that the material might come to a state of moisture-equilibrium with the air. The thimbles were then weighed and the material was quantitatively transferred to a mortar and ground to a fine powder. The starch was determined as glucose by the acid-hydrolysis method (Wiley, H. W., et al., 1908) in two accurately weighed fractions of this powder, each representing about one-half of the extracted residue before it was ground.

SUGAR.—For the determinations of sugar, samples of 25 gm. were washed into 250 c. c. volumetric flasks with enough neutral 70 per cent alcohol to bring the volume up to about 200 c. c. About 1 gm. of calcium carbonate was added to each flask. The flasks were then boiled in the water bath for 10 minutes and on the following day were cooled to 20° C. and filled to the mark. After being stoppered they were allowed to stand for a few days, during which they were occasionally shaken to insure uniformity of concentration of sugars in the solid and the liquid portions of the contents. The solutions were subsequently treated essentially according to the method described by Bryan, Given, and Straughn (1911). Reducing sugars and total sugars were determined according to the method of Allihn (Wiley, H. W., et al., 1908). The cane sugar was calculated from the difference between the total sugar and the reducing sugars.

TEMPERATURE.—The temperature of the two storage rooms was recorded by thermographs. The curves obtained in the warm storage room were integrated with a planimeter to obtain the average weekly temperatures, which are given in Tables I and II. The average weekly temperatures for the cold-storage room were written down from inspection of the records, since the tracings in this case were practically straight lines.

EXPERIMENTAL DATA

The data showing the seasonal changes in the composition of sweet potatoes stored in the farm cellar at a temperature varying mostly from 11.7° to 16.7° C. are given in Table I. The percentages of carbohydrates have all been referred to the original moisture content of the potatoes. The loss of solid matter by respiration had, of course, to be disregarded. The numbers expressing the total content of carbohydrates were obtained by the addition of the numbers representing the starch (as glucose) and the total sugars (as glucose).

TABLE I.—*Carbohydrate transformations in sweet potatoes stored in the farm cellar*

BIG STEM

Date.	Water.	Starch.	Cane sugar.	Reducing sugar as glucose.	Total sugar as glucose.	Total carbohydrates as glucose.	Gain or loss of starch (as glucose).[a]	Gain or loss of sugar (as glucose).[a]	Average weekly temperature.	
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Gm.*	*Gm.*	°C.	*Week ending—*
									26.7	Oct. 30
									21.7	Nov. 6
Oct. 20	73.50	19.07	1.90	0.90	2.90	24.09	.....	.....	16.7	Nov. 13
									16.7	Nov. 20
Nov. 8	72.99	16.94	3.51	1.32	5.02	23.85	−2.36	+2.12	15.6	Nov. 27
									15.0	Dec. 4
Dec. 6	71.89	16.42	3.94	1.40	5.55	23.79	− .58	+ .53	15.0	Dec. 11
									18.3	Dec. 18
									15.6	Dec. 25
Jan. 4	72.06	16.02	4.39	1.28	5.90	23.70	− .44	+ .35	14.4	Jan. 1
									12.8	Jan. 8
									11.7	Jan. 15
									12.8	Jan. 22
Feb. 1	72.18	14.11	6.06	1.67	8.04	23.71	−2.12	+2.14	16.1	Jan. 28
									15.0	Feb. 5
									11.7	Feb. 12
									[b] 12.2	Feb. 19
Mar. 1	71.97	13.09	6.96	1.44	8.76	23.31	−1.13	+ .72	16.7	Feb. 26
									14.4	Mar. 4
									[b] 15.0	Mar. 11
Mar. 20	73.02	13.44	6.40	1.10	7.84	22.77	+ .39	− .92	15.0	Mar. 18
									16.1	Mar. 25
Mar. 26	72.49	14.47	5.61	.87	6.77	22.85	+1.14	−1.07	16.1	Apr. 1
									18.9	Apr. 8
Apr. 16	72.87	14.20	6.03	.90	7.24	23.02	− .30	+ .47	16.7	Apr. 15
									17.8	Apr. 22
									20.6	Apr. 29
									18.9	May 6
June 1	72.45	14.62	5.85	.87	7.02	23.27	+ .47	− .22	18.9	May 13
									17.2	May 20
									21.1	May 27
									21.1	June 3

SOUTHERN QUEEN

									26.7	Oct. 30
									21.7	Nov. 6
Oct. 23	71.69	22.09	1.19	0.39	1.64	26.18	.....	.....	16.7	Nov. 13
									16.7	Nov. 20
Nov. 10	68.41	19.87	2.97	.77	3.89	25.96	−2.47	+2.25	15.6	Nov. 27
									15.0	Dec. 4
									15.0	Dec. 11
									18.3	Dec. 18
Dec. 7	67.69	19.30	3.50	.72	4.41	25.85	− .63	+ .52	15.6	Dec. 25
									14.4	Jan. 1
									12.8	Jan. 8
Jan. 11	67.51	19.75	3.53	.75	4.46	26.41	+ .50	+ .05	11.7	Jan. 15
									12.8	Jan. 22
									16.1	Jan. 28
Feb. 3	68.02	19.22	3.95	.60	4.75	26.11	− .59	+ .29	15.0	Feb. 5
									11.7	Feb. 12
									[b] 12.2	Feb. 19
Feb. 28	68.00	18.99	4.05	.53	4.80	25.90	− .26	+ .05	16.7	Feb. 26
									14.4	Mar. 4
									[b] 15.0	Mar. 11
									15.0	Mar. 18
Apr. 8	66.71	20.35	2.93	.52	3.61	26.22	+1.51	−1.19	16.1	Mar. 25
									16.1	Apr. 1
									18.9	Apr. 8
									16.7	Apr. 15
May 4	69.21	19.78	3.39	.51	4.07	26.05	− .63	+ .46	17.8	Apr. 22
									20.6	Apr. 29
									18.9	May 6
									18.9	May 13
June 4	68.15	20.15	2.80	.55	3.50	25.89	+ .41	− .57	17.2	May 20
									21.1	May 27
									21.1	June 3

[a] Per 100 gm. of material.

[b] Record obtained for only one day of this week.

The data in Table I show that under the conditions of this experiment the moisture content of the roots remains fairly constant. There is a slight decrease in the moisture content, more marked in the Southern Queen than in the Big Stem variety, during the curing process, but on the whole there is comparatively little change in the percentage of moisture. The loss of moisture is probably compensated in part by the water formed by respiration, while the loss of substance by respiration would increase the relative moisture content, thus tending to conceal actual water lost.

The percentage of starch shows a rather sudden decrease immediately after the potatoes are dug. The subsequent decrease is more gradual, and continues until a minimum is reached in March. After that time there is a continuous rise in the percentage of starch until the last date on which the potatoes were examined.

Concomitant with the changes in the percentage of starch there is an inverse change in the percentage of sugar. Corresponding with the first sudden decrease of starch, there is an equally sudden increase in sugar. Later the increase in sugar content is more gradual, and reaches a maximum at the time of the starch minimum. After the sugar content has reached a maximum there is a gradual decrease, which, however, is not as marked as the increase during the first part of the season.

The course of the changes in the percentage of cane sugar follows that of the total sugar in both varieties, but in the Southern Queen the invert sugar after the initial rise shows an almost continuous decrease, whereas in the Big Stem the invert sugar content also shows a distinct maximum.

The total carbohydrate content in both types remains fairly constant; consequently the numbers showing the loss (or gain) of starch between the successive dates of sampling show a fairly close agreement with those showing the corresponding gain (or loss) of total sugar. The aberrations are probably to be attributed partly to the loss of substance through respiration, but mostly to nonconformity of samples.

The data showing the carbohydrate transformation in sweet potatoes stored at low temperatures (approximately 4° C.) are given in Table II.

TABLE II.—*Carbohydrate transformations in sweet potatoes in cold storage*

BIG STEM, FIRST LOT[a]

Date.	Water.	Starch.	Cane sugar.	Reducing sugar as glucose.	Total sugar as glucose.	Total carbohydrates as glucose.	Gain or loss of starch (as glucose).[b]	Gain or loss of sugar (as glucose).[b]	Average weekly temperature.	
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Gm.*	*Gm.*	*°C.*	*Week ending—*
									7.8	Oct. 23
Nov. 8	72.99	16.94	3.51	1.32	5.02	23.85	.....	.....	7.2	Oct. 30
									5.6	Nov. 6
									5.6	Nov. 13
									4.4	Nov. 20
Dec. 9	72.99	13.31	6.46	2.02	8.82	23.62	−4.03	+3.80	4.4	Nov. 27
									4.4	Dec. 4
									3.9	Dec. 11
Dec. 21	70.77	10.80	7.33	1.60	9.31	21.31	−2.79	+ .49	3.3	Dec. 18
									2.8	Dec. 25

[a] The figures are all calculated for the original water content of the roots, 73.50 per cent.

[b] Per 100 gm. of material.

TABLE II.—*Carbohydrate transformations in sweet potatoes in cold storage*—Continued

BIG STEM, SECOND LOT[a]

Date.	Water.	Starch.	Cane sugar.	Reducing sugar as glucose.	Total sugar as glucose.	Total carbohydrates as glucose.	Gain or loss of starch (as glucose).	Gain or loss of sugar (as glucose).	Average weekly temperature.	
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Gm.*	*Gm.*	*°C.*	*Week ending—*
Mar. 27	72.19	12.99	6.41	1.65	8.39	22.82	.....	.....	.....	.....
Apr. 30	73.32	9.74	8.74	2.44	11.64	22.47	−3.61	+3.25	.....	.....

SOUTHERN QUEEN[b]

									7.8	Oct. 23
									7.2	Oct. 30
									5.6	Nov. 6
									5.6	Nov. 13
									4.4	Nov. 20
Nov. 10	68.41	19.87	2.97	0.77	3.89	25.96	.....	.....	4.4	Nov. 27
									4.4	Dec. 4
									3.9	Dec. 11
Dec. 8	66.77	17.40	5.93	.59	6.83	26.16	−2.74	+2.94	3.3	Dec. 18
Dec. 22	67.57	16.48	6.94	.65	7.96	26.28	−1.02	+1.13	2.8	Dec. 25

[a] The figures are all calculated for the original water content of the roots, 73.50 per cent.
[b] The figures are all calculated for the original water content of the roots, 71.69 per cent.

In these experiments three lots of potatoes were used. One lot of the Big Stem and one of the Southern Queen were placed in cold storage immediately after they had been cured. Another lot of the Big Stem variety which had been kept in warm storage until March 27 was placed in cold storage on that date. The cold-storage experiments were of short duration, since the potatoes invariably rotted after having been kept at the low temperature for about six weeks.

These data show that at low temperatures the disappearance of starch and the accumulation of sugar in sweet potatoes take place more rapidly and proceed to a greater extent than at high temperatures. As to the relative proportion of the individual sugars, the two types of potatoes seem to differ somewhat. In both types the cane-sugar content is markedly higher in cold than in warm storage. In the Big Stem sweet potatoes the invert-sugar content also is higher in cold storage, but in the Southern Queen the invert-sugar content is no higher in cold than in warm storage. In general, cane sugar is the chief product which accumulates at low temperatures. The total carbohydrate content, with one exception, remains fairly constant, and the increase of sugar accounts for the loss of starch. The exception mentioned is the discrepancy between the loss of starch and the gain of sugar in the Big Stem potatoes during the interval from December 9 to December 21. The only explanations that can at present be suggested for this discrepancy are either that after long exposure to low temperatures the various phases in the process of the transformation of starch into sugar are influenced in such a way that intermediate products which escape detec

tion by the analytical methods employed accumulate to a greater extent than usual; or, inasmuch as many of the potatoes showed small rotten spots at the time of the last sampling, it is possible that although these were cut out and the flesh appeared otherwise entirely sound, the enzyms secreted by the fungus had brought about a partial transformation of starch beyond the zone actually invaded by the mycelium.

DISCUSSION OF RESULTS

A striking fact brought out in the tables is the high starch content and the low sugar content of the sweet potato immediately after harvesting. A number of analyses, not here reported, of potatoes dug at different times also showed that freshly dug potatoes contain only small quantities of sugar. However, as soon as the potatoes are dug, a rapid transformation of starch into sugar takes place. A number of experiments not given here showed that this sudden transformation of carbohydrates takes place over a wide range of temperatures and that even at 30° C. the process is so rapid that sugar accumulates in excess of the quantity used in respiration, while at any subsequent period the accumulated sugar diminishes at that temperature as a result of respiration. This initial transformation is in such striking contrast with the later less rapid transformation that the two may almost be considered as distinct phases in the carbohydrate metabolism of the roots. It appears that during the period of active growth processes occur which prevent the accumulation of sugar in the roots. The elaborated materials from the leaves are almost wholly transformed into starch. The reverse process, which takes place as soon as the potatoes are dug, seems to be associated with the cessation of the flow of materials from the vines to the roots. The influx of materials from the vines therefore seems to determine the direction of the carbohydrate transformation in the growing roots.

Subsequent to the initial period, the carbohydrate transformations in the sweet potato are greatly influenced by temperature. In warm storage there is a continual accumulation of sugar in excess of the quantity used for respiration during the first part of the storage period. The corresponding disappearance of starch leaves no doubt as to the source of the sugar.

During the latter part of the season the process is apparently reversed. The increase in the percentage of starch and the decrease in the percentage of sugar during this period suggests that during the latter half of the storage season a re-formation of starch takes place, such as has been observed in twigs and woody stems and in the tubers of the common potato.

It should be noted, however, that increased respiration during the latter half of the season, during which the temperature of the storage room rose gradually, may account for the loss of sugar. However, the constancy of the total carbohydrates and the increase in the percentage of starch seem

to show that there is an actual transformation of sugar to starch during this period. In a general way the course of the carbohydrate transformations in sweet potatoes seems to be correlated with the seasonal variation in the temperature of the storage room. That the temperature may be the controlling factor in determining the direction of the carbohydrate transformation is shown by the continuous transformation of starch into sugar in the sweet potato as well as in other storage organs of plants at low temperatures and the reversion of the process at higher temperatures. Further experimentation is necessary, however, in order to determine whether temperature is the sole controlling factor.

In some respects the behavior of the sweet potato is in marked contrast to the behavior of resting storage organs of plants of temperate regions. In general, it has been found that the accumulation of sugar as a result of starch transformation ceases at temperatures only a few degrees above 0° C. Thus, Fischer (1891) found that in the cortex of trees the regeneration of starch takes place at a temperature a few degrees above 0° C., while Müller-Thurgau (1882) found that in the common potato the accumulation of sugar practically ceases at 8° C. In the sweet potato a rapid transformation of starch into sugar in excess of the quantity used for respiration takes place in freshly dug potatoes at temperatures as high as 30° C. At later periods a marked accumulation of sugar takes place in the sweet potato at temperatures much higher than those at which the accumulation of sugar ordinarily ceases in resting storage organs.[1]

The sweet-potato roots exhibit a further peculiarity with respect to the quantitative relations of the substances formed by the conversion of starch. With the exception of soft-wooded trees, where oil results from the conversion of starch, reducing sugars have been observed as the most usual and most abundant products resulting from starch transformation in resting storage organs. In the common potato Müller-Thurgau (1877) found that cane sugar is present together with glucose in the proportion of 1 part of cane sugar to 2.5 parts of glucose, while Appleman (1914) reports in potatoes kept at a temperature around 0° C. for 2½ months 3.94 per cent of total sugar and 2.40 per cent of reducing sugar. In the sweet potato cane sugar is the principal product formed by the conversion of starch, while the quantity of reducing sugar is small. In warm storage the cane-sugar content of the Big Stem sweet potatoes reached 6.96 per cent and that of the Southern Queen 4.05 per cent, while the maximum reducing sugar contents were, respectively, 1.67 and 0.77 per cent. In cold storage the cane-sugar content of the two types rose to 8.74 and 6.94 per cent, respectively, while the maximum reducing sugar content in the two cases was 2.44

[1] The transitory solution initiating the process of translocation of starch in leaves and in the storage organs of plants about to resume active growth, of course, takes place at higher temperatures. This process seems to be somewhat different in its nature from the solution of starch in resting storage organs as a result of exposure to low temperatures

and 0.77 per cent, the invert sugar content of the Southern Queen having shown no increase in cold storage. In all cases the proportion was approximately 4 to 5 parts of cane sugar to 1 of reducing sugar.

SUMMARY

During its growth the sweet-potato root is characterized by a very low sugar content. The reserve materials from the vines are almost wholly deposited as starch.

Immediately after the roots are harvested there occurs a rapid transformation of starch into cane sugar and reducing sugars. This initial transformation seems to be due to internal causes and is largely independent of external conditions. Even at a temperature of 30° C. both cane sugar and reducing sugars accumulate during this initial period in excess of the quantity used in respiration, while during subsequent periods the quantity of reducing sugar diminishes at that temperature as a result of respiration. These initial changes seem to be associated with the cessation of the flow of materials from the vines.

In sweet potatoes stored at a temperature of 11.7° to 16.7° C., the moisture content remains fairly constant. There is a gradual disappearance of starch during the first of the season (October to March) and probably a re-formation of starch accompanied by a disappearance of cane sugar during the latter part of the season (March to June). The changes in reducing sugar are less marked than those in cane sugar. The changes in starch and cane sugar appear in a general way to be correlated with the seasonal changes in the temperature.

In sweet potatoes kept in cold storage (4° C.) there is a rapid disappearance of the starch and an accompanying increase in cane sugar. These changes do not attain a state of equilibrium at that temperature, as the sweet potatoes invariably rot by the action of fungi before the changes have reached their maximum. At both high and low temperatures cane sugar is the chief product formed by the conversion of starch in the sweet potato. The quantity of invert sugar in the root at any time is comparatively small.

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PRELIMINARY AND MINOR PAPERS

THREE-CORNERED ALFALFA HOPPER

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INTRODUCTION

The small triangular insect of the hemipterous family Membracidae on which this paper is based was first noted and described as *Membracis festina* by Thomas Say in 1831 (1);[1] and in 1869 Stål (2, 3) referred it to the genus Stictocephala. Since that time it has frequently been noted by entomological writers, who usually merely mentioned its occurrence in a new locality or repeated what had already been observed. In 1888 this insect was first noted in literature as being injurious (4). However, the species was not generally considered of economic importance until the winter of 1910, when Prof. Herbert Osborn, in a paper (11) read before the American Association of Economic Entomologists, called attention to the economic habits of the genus Stictocephala and gave special attention to the species *S. festina* and its economic relation to alfalfa and clover.

Prof. Osborn in his paper stated that little or nothing was known of the life history and habits of the species. It is the purpose of this paper to give a report of the same, together with other related data, as collected by the writer, assisted by Messrs. R. N. Wilson and T. Scott Wilson at Tempe, Ariz., and by Mr. Edmund H. Gibson at Greenwood, Miss.

SPECIFIC IDENTITY OF THE THREE-CORNERED ALFALFA HOPPER

The name "three-cornered alfalfa hopper," adopted for this insect because it is the common term applied to it by farmers throughout areas of heavy infestation, is applicable to both Say's (1) *Stictocephala festina* and Van Duzee's (10) *Stictocephala festina*, var. *rufivitta*. On several occasions Mr. Otto Heidemann has determined a few specimens as *S. festina*, var. *rufivitta*, among material sent to the Bureau of Entomology for identification. As these were secured both by Mr. Gibson in Tennessee and Mississippi and by the writer in Arizona, one is led to believe that the species and the so-called variety occur rather generally together. Since Van Duzee (10) bases his description of the *rufivitta* variety upon male specimens only, and since only male specimens among hundreds examined have exhibited the determining character—namely, that "the dorsal carinæ are not evanescent before their point of meeting the

[1] Reference is made by number to "Literature cited," p. 362.

posterior carinæ," both the writer and Mr. Gibson, who has made many observations on this point, feel that this variety has been founded on too slender grounds.

Van Duzee's variety *angulata* has never been taken.

DISTRIBUTION

Osborn, in his paper (11) on the genus Stictocephala, points out that *S. festina* has a wide distribution and is found throughout the southern and southwestern United States. It is certain that in these sections it occurs in the greatest abundance, but its range is not limited to them. (See fig. 1.) Say (1) described the species from specimens secured in Florida. In 1889 Provancher (5) reported the species in Ottawa, Canada, and in 1890 Smith (6) gave New Jersey as a new locality. Later

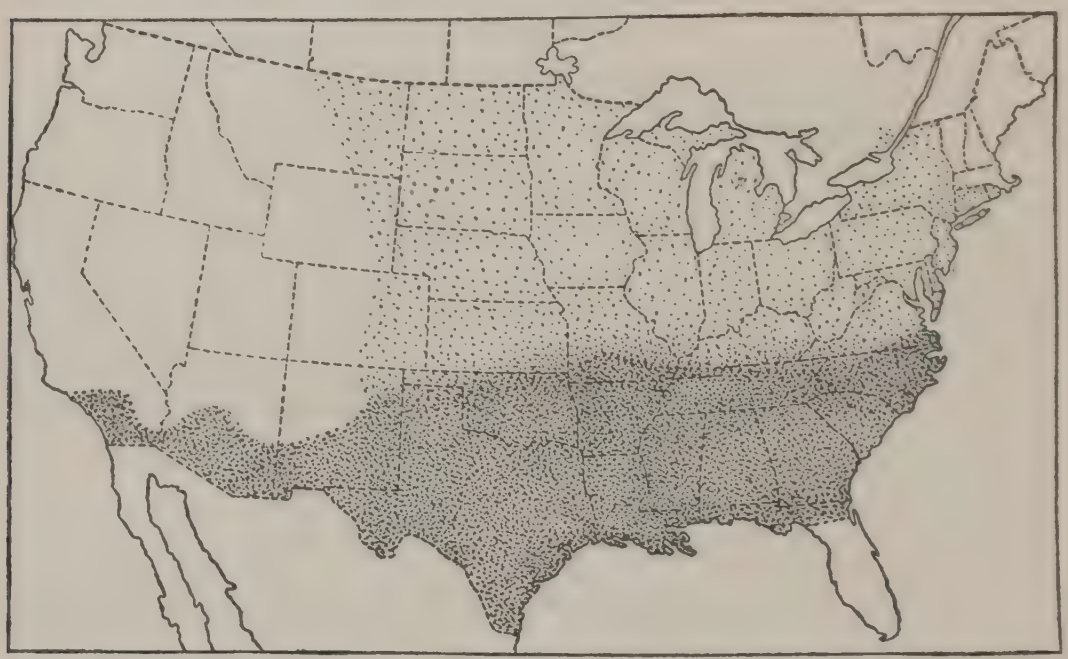

FIG. 1.—Map showing distribution of the three-cornered alfalfa hopper (*Stictocephala festina*) in the United States. The densely dotted area shows region of injurious infestation; the sparsely dotted area shows region of occurrence in limited numbers. Original.

than this (1894) F. W. Goding (8) gave the following localities: Virginia, Pennsylvania, Georgia, Florida, Missouri, Texas, Iowa, Montana, and Colorado (Riley); New York and Connecticut (Van Duzee); New Jersey (Smith); Canada (Provancher). That the species occurs in very limited numbers in the northern half of the United States is certain. Osborn found in his travels of 1909 and 1910 that *S. lutea* has a southern boundary agreeing quite well with the northern boundary of *S. festina*. The writer had the pleasure of examining alfalfa sweepings made by Mr. R. N. Wilson at 12 different localities in Colorado and Utah during the summer of 1911, and although several species of Membracidae were represented in the collections, not one specimen of *S. festina* was present.

The writer has observed the species in abundance throughout the Southwestern States, and found it injuring alfalfa (*Medicago sativa*) at Yuma, Tucson, Casa Grande, Tempe, Phoenix, Buckeye, and Glendale, Ariz., while Mr. R. N. Wilson reported it in alfalfa sweepings at Sacaton, Ariz., a region

isolated by a desert from any other cultivated area. Specimens were taken by the writer at Bard, Cal., another region remote from cultivated areas. Throughout the Imperial Valley in southern California the alfalfa hoppers were found in injurious abundance, while in Mexico, in the peninsula of Lower California, the pest was taken in numbers. In 1912 and 1913 Mr. Edmund H. Gibson found the species well distributed throughout the States of Mississippi and Tennessee and in several localities in Alabama, as well as at Atlanta, Ga.

EFFECT OF ALTITUDE ON DISTRIBUTION

It is quite interesting to note here that in Arizona and New Mexico the species is distinctly one inhabiting lower altitudes. During the summer of 1913 a great many observations were made on this point. The highest point, to the writer's knowledge, at which it has been taken is Fairbank, Ariz., where, at an altitude of 3,868 feet, on September 4, 1913, Mr. Harry Newton, then an agent of the Bureau of Entomology, found both nymphs and adults to be quite common on alfalfa. The writer made sweepings at Raton, Cimarron, and Las Vegas, N. Mex., all at an altitude above 5,000 feet, and while many alfalfa insects common in lower altitudes were taken, not a specimen of Stictocephala was secured. At Ute Park, Taos, Embudo, Bluewater, and Gallup, N. Mex., localities ranging in altitude from 5,000 to 8,000 feet, Mr. J. R. Sandige made sweepings from alfalfa and likewise failed to take a single specimen of Stictocephala, although it is known to occur in lower altitudes in the State. Possibly the most striking observations were those of Mr. R. N. Wilson, made while on a trip through Arizona for the express purpose of securing records on this species. He visited points varying in altitude from 2,000 to 7,000 feet, but never found the species above about 3,000 feet. His note, made on August 25, 1913, giving a summary of the trip, is as follows:

> The writer returned to-day from a trip over part of Arizona, including stops at Prescott, Camp Verde, Williams, Show Low, Pinetop, White River, and Gila River Valley points from Rice to Solomonsville and Miami. Special alfalfa sweepings were made at each of the above-mentioned places to determine whether or not *Stictocephala festina* occurred in that locality. The highest altitude at which the species was found was 3,000 feet, at Camp Verde. Altitudes varying from this to 7,000 feet were examined, but no trace of *S. festina* was found. When Rice was reached, where the altitude is only 2,500 feet, this species was again found in numbers, and all the way up the Gila River Valley to Solomonsville (altitude 2,985 feet) the Stictocephala were very common.

FOOD PLANTS

The alfalfa hopper lives on a great variety of food plants. Its general distribution and the fact that it is found in such isolated places under cultivation are doubtless due to the wide range of its food habits and probably, also, to the presence of native leguminous plants upon which, in all probability, it lives. Its favorite foods without a doubt belong to the legume family, for it is particularly fond of alfalfa, cowpeas (*Vigna sinensis*), and the various clovers, but it has also been found feeding upon trees, shrubs, herbs, and grasses.

The earliest recorded food plant is the tomato, which in 1888 was reported by Dr. Oemler (4) as being injured by this species. The next record we have is Prof. Cockerell's (9) in 1899, when he reported the hopper as feeding on alfalfa. He also mentions its occurrence on almond

trees, but does not say whether it was feeding thereon or not. Prof. Osborn (11) reported it in 1910 as feeding upon both alfalfa and clover. The writer has found the species feeding as well as breeding on Bermuda grass (*Capriola dactylon*), Johnson grass (*Sorghum halepense*), wheat (*Triticum* spp.), barley (*Hordeum sativum*), oats (*Avena sativa*), bur clover (*Medicago denticulata*), yellow sweet clover (*Melilotus officinalis*), and alfalfa, which, as has been stated, is its principal food plant.

Mr. T. Scott Wilson took specimens feeding on soy bean (*Glycine hispida*) at Sacaton, Ariz., and Mr. Edmund H. Gibson, besides reporting the species as feeding upon alfalfa, also finds it feeding upon vetch and *Hordeum murinum* at Tempe, Ariz., and upon red clover and cowpeas at Greenwood, Miss., and in fact doing its greatest damage to the last-named plant. Dr. A. W. Morrill, State Entomologist of Arizona, has found the insect feeding upon beans and in some instances proving a pest to that plant. Late in the season one finds the insect resting upon many varieties of plants, but whether feeding on all these is unknown. Mr. R. N. Wilson found it upon the following plants: Sunflower, upon which it was doubtless feeding; cocklebur; *Atriplex truncata; Erigeron canadensis* and *Erigeron* sp.; mesquite and cottonwood, feeding on the former; *Sporobolus airoides*, and *Trichlaris mendocina*.

DESCRIPTION OF THE THREE-CORNERED ALFALFA HOPPER

THE ADULT

The adults (Pl. XLIII, fig. 1) are about 6.16 mm. long and light green in color. The accompanying table of measurements (Table I) made by Mr. Gibson shows that the males are slightly smaller than the females.

TABLE I.—*Length of live adults of the three-cornered alfalfa hopper*

Specimen No.	Length of—	
	Male.	Female.
	Mm.	*Mm.*
1	6.0	6.0
2	6.4	6.3
3	6.1	6.3
4	5.9	6.0
5	6.0	6.6
Average length [a]	6.8	6.24

[a] Average length of 10 adults, 5 males and 5 females, is 6.16 mm.

The males have a reddish line down the dorsum of the prothoracic shield. This marking, being absent in the female, is a sex character by which mature males and females are quite readily distinguishable. The insects are triangular in shape, presenting a broad solid aspect when viewed from the front (Pl. XLIII, fig. 1, *b*). The following original description, made by Thomas Say (1) in 1831, was evidently made from male specimens, because, as is mentioned above, the females do not have the "carina tinged with rufous."

> Thorax with a subacute line each side before, meeting behind the middle. Inhabits Florida.

Body yellowish-green: thorax unarmed, carinate behind; at tip attenuated, subulate and complying with the general curvature; each side before a carinate line, meeting together at the carina behind the middle, with the carina tinged with rufous; front of the thorax not altogether flat, but a little convex; hemelytra, three terminal cellules unequal; the two costal ones equal, as broad as long; the inner one not obviously larger than the others together, somewhat longer than broad. Length to tip of hemelytra one fifth of inch. The lateral prominent lines of the unarmed thorax, separate this species from all those I have described excepting goniphera, which, meet before the middle of the length of the back.

THE EGG

The egg (Pl. XLIII, fig. 2, *b*) is about 1 mm. (0.9 to 1.3 mm.) long and 0.35 mm. (0.25 to 0.4 mm.) in diameter. It is white, rather oblong, slightly larger at one end, and with a greater curve on one side. The surface is smooth, except a portion on the larger end which is regularly covered with small papillæ.

THE NYMPH

The nymphs are the same general shape as the adults, but instead of having the prothoracic shield as a body covering, they are regularly covered with prominent projections, spines, and hairs. There is one dorsal pair of these projections on the head, four pairs on the thorax, and seven pairs on the abdomen, the posterior pair on the anal segment being much reduced in size.

Their general color is as follows: Head and thorax very light straw. Eyes with margin white and center cologne earth. Antennæ white. Thorax with a regular cologne-earth patch on each side, widest on the mesothorax, where it reaches the darkest shade. Legs white, except tip of last tarsal joint, which is dark brown. Abdomen white, approaching light green, owing to food material within, with the irregular dark spot of the thorax extending narrowly across the first segment, widening greatly on segments 2, 3, and 4, and showing only on the posterior margin of the fifth, being widest on the posterior margin of segments 3 and 4. Anal segment light-straw color at extreme end.

The different nymphal stages, of which there are five, are the same in general appearance, except that the main dorsal projections in the first stage have only one subspine, while in the second and remaining stages there are numerous branches. A second difference is the growth posteriorly of the prothoracic shield and the appearance of wing pads in the last three stages. These two differences, with the increase in the size of the body and the general darkening of colors in each successive stage, enable one to recognize any of the different stages.

DESCRIPTION OF INDIVIDUAL STAGES

STAGE I (Pl. XLIII, fig. 2, *a*).—Length, 1.6 mm. (1.4 to 1.7 mm.), average of 10 specimens. Head, thorax, abdomen, and all appendages pale when first born. After feeding the abdomen takes on straw color and the cologne-earth patch of the thorax becomes faintly visible. Eyes white. Twelve pairs of dorsal hairlike projections with one upright spine. One pair on head, four on thorax (two on prothorax, one each on mesothorax and metathorax), and seven on abdomen. Spines colorless, pale. Body regularly but sparingly covered with spines, conspicuous and large compared to size of body.

STAGE II (Pl. XLIII, fig. 3).—Length, 2.1 mm. (1.9 to 2.5 mm.), average of 10 specimens. Head, thorax, abdomen, and appendages light straw colored. The cologne-earth patch of the thorax becomes more pronounced and extends back onto the abdomen. Eyes in this and succeeding stages with white margin and brown center. The dorsal projections have become fleshy and bear several lateral spines, the upright spine

being reduced in length. Other body spines much more numerous than in first stage, but reduced in proportionate size.

STAGE III (Pl. XLIII, fig. 4).—Length, 2.9 mm. (2.6 to 3 mm.), average of 10 specimens. Head, thorax, abdomen, and appendages dark straw color, cologne-earth patch in some specimens especially pronounced. Dorsal projections more fleshy and containing a greater number of lateral spines. Prothoracic shield beginning to develop. Wing pads faintly visible.

STAGE IV (Pl. XLIII, fig. 5).—Length, 3.8 mm. (3.5 to 4.1 mm.), average of 10 specimens. Head, thorax, abdomen, and appendages greenish straw color. Dark patch on thorax and abdomen becoming dark brown, almost black. The color varies greatly, however, some specimens being light green and others very dark throughout the stage. Dorsal projections in this and fifth stage quite fleshy, lateral spines numerous. Prothoracic shield with posterior projection extending nearly to end of thorax. Wing pads clearly defined.

STAGE V (Pl. XLIII, fig. 6).—Length, 4.8 mm. (4.5 to 5 mm.), average of 10 specimens. Color same as in Stage IV, about the only difference between this stage and Stage IV being the enlarged size. Point of prothoracic shield extending over the first segment of the abdomen and wing pads extending to posterior part of second abdominal segment.

LIFE HISTORY AND HABITS

The observations on the three-cornered alfalfa hopper have been carried through two years and parts of two others at Tempe, Ariz., and through one entire year at Greenwood, Miss. The results, therefore, have been secured under widely differing conditions, the former place being in a hot, semiarid country with an annual rainfall of about 8 inches, while the latter is in a warm, humid country with an average annual rainfall of nearly 50 inches.

In the Salt River Valley of Arizona much difficulty was experienced in securing life-history records during the months of June, July, and August, because of the excessive heat. In order to have the specimens under close observation, it was, of course, necessary to confine them in cages under more or less artificial conditions, and under such conditions the death rate among nymphs was very high. The combined lengths of the egg and nymphal stages under Arizona conditions varied with the temperature, being from 35 to 114 days, with an average of about 50 days for all conditions. In Mississippi Mr. Gibson found that a much shorter period was required. Here the variation, as shown by observations on a much smaller number of specimens, was from 26 to 37 days. Records of a larger number of specimens would doubtless have given a wider variation.

EGG STAGE

In Arizona the egg stage varies from a minimum of 12 days to a maximum of 41 days, this variation depending upon the prevailing temperature. The average for all records is 22 days. As may be seen in Table II, during an average mean temperature of 59° F. the time required for incubation varied from 33 to 43 days. At a mean temperature of 63° the variation was from 23 to 30 days, while with a mean temperature of about 85° the eggs hatched in a period ranging from 12 to 17 days.

TABLE II.—*Length of the egg stage of the three-cornered alfalfa hopper at Tempe, Ariz., in 1912; host, alfalfa*

Cage No.	Eggs laid.		Eggs hatched.		Length of incubation.	Temperature.
	Date.	Number.	Date.	Number.		
					Days.	°*F.*
1[a]	Feb. 6	2	Mar. 7	2	30	.....
5[a]	6	1	7	1	30	.....
9	Mar. 19	[b] Many.	Apr. 22	2	33	[c] 59
			23	1	34	
			25	1	36	
			May 1	6	43	
10	22	Many.	Apr. 25	2	34	[c] 59
			26	4	35	
			27	3	36	
			May 1	3	40	
			2	4	41	
16	Apr. 9	Many.	3	1	24	63
			5	1	26	
17	10	Many.	3	1	23	63
			4	0	.....	
			5	2	25	
			6	4	26	
			7	2	27	
			8	3	28	
			9	1	29	
			10	2	30	
24	May 24	Many.	June 5	28	13	83
			6	25	14	
			7	49	15	
			8	12	16	
25	25	Many.	6	3	12	84
			7	13	13	
			8	4	14	
			10	1	16	
37	June 21	Many.	July 6	7	15	85
			7	3	16	
			8	2	17	
38	July 5	Many.	20	9	15	87
			21	3	16	
			22	2	17	

[a] Influenced by artificial heat.
[b] No way of getting exact count without disturbing the eggs.
[c] Average mean temperature.

At Greenwood, Miss., Mr. Gibson was able to get eggs to hatch in the remarkably short time of four days. On June 30 eggs were deposited in cowpea stems, and on July 3 several had hatched. The writer is unable to surmise the reason for this short duration of the incubation period. The temperature, although no records are available, could hardly have been higher than that recorded during July at Tempe. The amount of humidity may have had something to do with the hasty incubation; then, too, the different host plant, all records from Tempe having been made on alfalfa, may have been a factor in lessening the period. Table III gives the results of Mr. Gibson's experiments to determine the length of the egg stage.

TABLE III.—*Length of the egg stage of the three-cornered alfalfa hopper at Greenwood, Miss.; host, cowpeas*

Date of oviposition of adults.	Date of egg hatching.	Length of egg stage.	Number of eggs.
		Days.	
June 30	July 3	4	10
Sept. 1	Sept. 8	7	Many
3	8	5	8

OVIPOSITION

IN ALFALFA.—The egg is deposited beneath the epidermis through a long slit made in the stem of alfalfa by the female with her ovipositor. This slit is often several times the length of the egg. Measurements of a large number of slits displayed a variation in length of from 0.75 to 2.25 mm. The egg is placed either just below or to one side of this puncture, and occasionally, instead of being just under the epidermis, an egg may be found shoved deep within the plant tissues, even to the center of the stem or beyond. Usually only one egg is deposited through a single opening, but sometimes two or more are placed together. Quite often, however, a great many slits are grouped side by side and the eggs laid singly but giving the appearance of having been bunched through the same opening. When this is the case, a large scar is made, and the place of oviposition would be quite noticeable if it were not for the fact that it usually occurs back of a sheath leaf or at the surface of the ground, where it is partially hidden. Females have been found with their abdomen extended down the stem of the plant and below the surface of the ground, and subsequently eggs have been found in the stems at such places. It has been observed that eggs are usually laid at night or early in the morning. These observations were made, however, during extremely warm weather; during cold weather the females would probably pick out the warmer part of the day to display their activities and thus avoid the minimum temperature, as they doubtless avoid extreme temperature.

IN COWPEAS.—The method of oviposition in cowpea stems is considerably different from that in alfalfa, the texture of the cowpea plant evidently making possible the placing of a great many eggs in the stem through one opening. Mr. Gibson has found that the eggs are always laid in groups and in his field notes quite aptly refers to these places of oviposition as egg pockets. He has observed from 1 to 12 eggs in a pocket. A count of the eggs in six pockets showed respectively 6, 4, 12, 2, 3, and 5 to the pocket. Following oviposition, in about one-third of these pockets a gall formation develops. From their appearance these must be similar to the galls which develop on alfalfa stems following ringing and which are described in the paragraph on alfalfa injury. These naturally give the egg pockets a distinctive and peculiar appearance. They are often as large as the stem itself, sometimes as much as one-fourth of an inch in diameter, and well show the efforts of the plant toward the healing of the injured part. Mr. Gibson thinks that these galls are due to an overproduction of epidermal cells caused by the physiological stimulus given to the plant by the injury, and states that they are of about the same texture and hardness

as the plant stem itself. It seems probable that the eggs in pockets where these galls have developed may be so interfered with that they can not incubate, but no definite observations were made on this point.

NYMPHAL PERIOD

The nymphal period comprises five stages, with a total length of from 22 to 69 days, depending upon the prevailing temperature. As is shown in Table IV, during the cooler spring month of March the total length varied from 42 to 69 days, while in the hot month of July the variation was from 22 to 37 days. The length of the different stages was found to be very unequal, the last two being found to average the longest, while the fifth might be prolonged almost indefinitely, provided food or other conditions were not right. This in itself is an important point, for the species would be able to survive for some time on only a minimum of food. There was found to be very little relation between the length of the periods and the sex of the individual.

TABLE IV.—*Lengths of the nymphal stages of the three-cornered alfalfa hopper at Tempe, Ariz., in 1912 and 1913*

Cage No.	Date of hatching of egg.	Date of first molt.	Length of Stage I.	Date of second molt.	Length of Stage II.	Date of third molt.	Length of Stage III.	Date of fourth molt.	Length of Stage IV.	Date of fifth molt.	Length of Stage V.	Total length of nymphal period.		Mean average temperature.
	1912.		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*	*Sex.*	*Days.*	°*F.*
1 [a]	Mar. 7	Mar. 17	10	Mar. 25	8	Apr. 4	10	Apr. 15	11	Apr. 28	13	♂	42	.....
3 [a]	7	17	10	24	7	3	10	14	11	30	16	.....	44	.....
4	7	19	12	Apr. 3	15	11	8	25	14	May 13	18	♀	67	61
4	7	20	13	3	14	11	8	25	14	13	18	♀♀	67	61
5	7	19	12	Mar. 29	10	8	10	19	11	15	26	♀♂	69	61
6	8	20	12	27	7	17	21	29	12	15	16	♀♂	68	61
12	27	Apr. 7	11	Apr. 14	7	21	7	29	8	17	18	♀♂	50	65+
15	27	7	11	15	8	21	7	29	8	25	25	♀♂	57	65+
21	May 1	May 9	8	May 14	5	May 20	6	June 1	12	June 12	11	♂	42	76+
22	1	7	6	13	6	19	6	May 29	10	14	16	♂	44	76
36	June 8	June 15	7	June 20	5	June 24	4	June 29	5	July 9	11	♂	32	84
36	8	16	8	21	5	25	4	July 1	5	10	9	♂	33	84
38	July 22	(*b*)	(*b*)	(*b*)	(*b*)	(*b*)	(*b*)	(*b*)	(*b*)	Aug. 28	(*b*)	2 ♂	37	86
39	6	(*c*)	(*c*)	(*c*)	(*c*)	(*c*)	(*c*)	(*c*)	(*c*)	8	(*c*)	♀ and 2 ♂	33	86
41	21	(*d*)	(*d*)	(*d*)	(*d*)	(*d*)	(*d*)	(*d*)	(*d*)	Aug. 22 and 23.	(*d*)	♂ and ♀	32 and 33	86
	1913.													
49	Apr. 12	Apr. 18	6	Apr. 24	6	Apr. 29	5	May 7	8	May 25	18	.....	43	69
50	12	17	5	24	7	29	5	9	10	27	18	.....	45	69
52	May 1	May 8	7	May 12	4	May 17	5	(*e*)	(*e*)	June 9	22	♂	39	73
53	1	8	7	13	5	18	5	27	9	9	13	.....	39	73
56	1	7	6	12	5	15	3	20	5	1	12	.....	31	71
57	1	8	7	13	5	19	6	24	5	8	15	♀♀	38	73
59	1	8	7	12	4	17	5	24	7	8	15	♀♀	38	73
60	2	8	6	12	4	(*e*)	(*e*)	21	9	May 28	7	.....	26	71
61	2	7	5	12	5	May 15	3	22	7	June 6	15	♀♀♂♂♂	35	73
62	2	8	7	12	4	17	5	24	7	14	21	♀♀♂♂♂	44	74
63	5	10	5	14	4	19	5	25	6	8	14	♀♂♂♂	34	72
67	5	9	4	13	4	18	5	26	8	14	19	♀♂♂♂	40	73
72	7	11	4	17	6	23	6	30	7	8	9	♀♂♂	32	75
73	7	17	10	21	4	27	6	(*e*)	(*e*)	8	12	♀♂♂	32	75
84 [f]	July 1	July 6	5	July 9	3	July 13	4	July 17	4	July 25	8	♂	25	87−
85 [f]	3	7	4	10	3	13	3	17	4	26	9	♂	23	86
86 [f]	1	5	4	8	3	12	4	16	4	23	7	♂	23	87+
87 [f]	1	5	4	9	4	13	4	18	5	27	9	♂	27	86

89 *f*		1	5	4	9	4	12	3	16	4	22	6			22	87+
90 *f*		2	7	5	10	5	14	4	18	4	28	10			26	87−
91 *f*		2	7	5	10	3	14	4	19	5	30	11			28	85+
Average				7.2		5.8		6		7.66		14.25			38.75	
Maximum				13		15		21		14		26				
Minimum				4		3		3		4		7				

[a] Influenced by artificial temperature.
[b] 2 specimens; records missing.
[c] 3 specimens; records missing.
[d] 2 specimens; records missing.
[e] Records missing.
[f] Records by T. Scott Wilson.

It is to be noted that in the third stage the 21-day maximum was observed in only one specimen, and this seems to be an extreme one, as the next highest maximum was only 10 days.

Looking at the Greenwood (Miss.) records for the nymphal periods, one finds not nearly as much variation between these and the records for Tempe, Ariz., as was exhibited in the incubation records for the two places. The nymphal period required from 22 to 30 days for completion with cowpeas as a host plant.

TABLE V.—*Length of nymphal period of the three-cornered alfalfa hopper at Greenwood, Miss.*

Date of emergence from egg.	Date of last molt.	Length of period.
		Days.
July 3	Aug. 3	30
Aug. 8	Sept. 3	26
Sept. 8	30	22
8	Oct. 6	28

HABITS OF THE NYMPHS

HATCHING.—The egg in hatching splits across one end and about one-fifth of the way down one side, and the nymph wriggles its way out. Its legs spread and in a few minutes it begins feeding. Two specimens were timed and one required 18 and the other 28 minutes to complete the process.

PROTECTION.—If left alone, the first-stage nymphs are very quiet and slow of movement, feeding in almost the same spot for days. As soon as approached by any object, they hastily place themselves on the other side of the plant and out of harm's way. The older nymphs are quite active and along with the younger exhibit a peculiar protective habit. If approached by an enemy or a supposed enemy, as the point of a camel's-hair brush, they throw the point of the abdomen toward the object and voiding a large bubble of watery excrement, explode it in the face of the enemy and then hastily move to the other side of the plant. In teasing a nymph in order to get it to display this habit the writer has cautiously moved the point of a lead pencil at the head of the nymph, and in trying to project the anal segment towards the pencil the nymph would nearly lose its footing. This habit is probably of considerable benefit as a protection and along with the horny appearance of the nymph doubtless furnishes immunity from many a hungry foe.

MOLTING.—The process of molting is interesting. As observed in two specimens it required 48 minutes for the one and 32 minutes for the other. Most of this time was occupied in getting a split started in the thorax. After the split was once started, the actual time required for the insects to wriggle out was 2 and 5 minutes, respectively. The description of the action is taken from the writer's original notes, made on March 20, 1912.

Just previous to molting, the skin becomes very tight and rigid, owing, of course, to pressure from within. The abdomen appears like an overinflated football bladder. The specimen, becoming quiet, forces its proboscis firmly into the stem, and using this as a pivot, with its legs to assist, it begins various body movements, such as straightening out its head, waving its abdomen up and down and, alternately with this, hunching the thorax upward, then resting a brief moment, whereupon the same

process is repeated. This was continued for three-quarters of an hour and then, after a minute's rest, with one final effort an opening was split on the dorsum of the entire thorax and head and the delicate white insect began to appear. The spines on the thorax were first pulled out, and then the insect continued its wriggling and gradually the abdomen was pulled from the old abdominal skin, the larva all the time working itself forward over the cast skin of the head. The legs were not entirely withdrawn until after the last segment of the abdomen was freed. The insect, then crawling the rest of the way over the head of the exuvia, came to rest on the plant just ahead of the cast skin. There it remained resting for 30 minutes, during which time the newly exposed tissue was becoming hard and firm and accustomed to the surrounding atmospheric conditions, after which the insect began feeding.

THE ADULT STAGE

The adults are strong, quick flyers. They are wary and, like the young, upon the approach of danger hastily move to the opposite side of a plant; then, as the enemy comes closer, with a spring they are off—how swiftly can only be appreciated by one who has been unexpectedly hit on the face or in the eye by an alfalfa hopper.

As observed in the cages kept for the purpose of determining the number of generations, the males are more numerous than the females, being in the proportion of 4 to 3. The female, after issuing from the last nymphal stage, requires from 8 to 10 days to complete her development. During this time she is feeding, and at the end of the period copulation takes place. A few days thereafter oviposition begins. The males die shortly after copulation, while the females lay eggs for a considerable period. Four hibernating females were placed in a cage on February 13, and on February 26 several eggs were laid; oviposition was continued until May 5, the females dying soon thereafter. Thus there was an egg-laying period of 70 days. A maximum of only 50 eggs was secured from a single female. The four mentioned above laid a total of 129 eggs, or an average of 32 each. It is possible, however, that these may have deposited eggs previous to entering hibernation. Eight other females deposited from 7 to 19 each. These numbers all seem small for maximums, but they were secured under unnatural cage conditions. Without much doubt a larger number than this, possibly as many as a hundred, are deposited by single females, when they are free and unhampered, as in the field.

SEASONAL HISTORY

HIBERNATION

The seasonal history of this species varies during different years, the variation quite naturally being due to the climatic conditions, especially the minimum temperature of any particular year. This variation appears largely during the winter months, when the species is supposed to be hibernating. At Tempe, Ariz., during a mild winter, such as the last one (1913–14), the species does not hibernate at all in the adult stage. Adult males and females were taken feeding on alfalfa every week during December, January, and February of last winter. Mr. Gibson, who made the January and February observations at Tempe, discovered that eggs deposited in the late fall months hatched on warm days, but the nymphs were usually killed during the cold nights following. During winters when the minimum temperatures are much lower the species goes into hibernation both as eggs and adults. During the winter of 1912–13 such conditions as those just mentioned were noted,

and because of the variation in minimum temperatures existing between the winter of 1912–13 and that of 1913–14 and its relation to hibernation, Table VI, showing the minimum temperatures, is given.

TABLE VI.—*Average minimum temperatures at Tempe, Ariz., during the winters of 1912–13 and 1913–14*

Period.	1912–13	1913–14
	°F.	°F.
Dec. 1 to 10	39	35
11 to 20	33	40
21 to 31	27.5	38
Jan. 1 to 11	24.5	40.3
10 to 20	32.7	43.5
21 to 31	33.7	40
Feb. 1 to 10	39.2	32.3
11 to 21	35.5	45.2
21 to 28	38	40
Average minimum for 3 months	33.6	39.4
Actual lowest temperature	12	29

While it will be noted in Table VI that there was a difference of nearly 6 degrees in the average minimum of the three months, December 1912, and January and February, 1913, as compared with the same three months of 1913–14, yet the most striking difference and the one that influenced hibernation is noted between December 11 and February 1 of the two years. For the former winter, the one during which the species hibernated, the average minimum from December 11 to January 31 was 30.3° F., and the actual lowest was 12° F., as against an average minimum of 40.3° F. and an actual lowest of 29° F. for the same period of the winter 1913–14, during which time the species was continually active.

During the winter of 1912–13 the hibernating period lasted about two months. Just how long it may last any other winter will depend upon the temperature; if, as was shown to be the case last winter, the temperature remains high enough, the insect will not go into hibernation. At Tempe the adults have been found particularly abundant at the base of bunch grass (*Sporobolus airoides*) and they are also found hiding below rubbish, leaves, etc., at the base of plants such as will provide them with green food on the first warm days of spring.

In Mississippi Mr. Gibson has found that the hibernating period lasts from December until March and that the chief protection for the dormant adults consists of bunches of *Andropogon* spp., in the clumps of which he has counted as many as 63 adults. At Nashville, Tenn., he has observed hibernating adults active by March 11.

SUMMER ACTIVITY

In Arizona hibernating adults that come forth during the first part of February deposit eggs soon thereafter, and during the latter part of March, April, and the first part of May die off. Young of the first generation, whether they come from overwintering eggs or from eggs of hibernating adults, appear during the month of March. There are from three

to four generations annually. During 1912 the writer observed three and a partial fourth, and during 1913 Mr. T. Scott Wilson observed the same number. The species reaches its greatest numbers during September, when adults of the third generation are appearing. Immediately following the first of November the adults begin disappearing quite rapidly. Many doubtless deposit their complement of eggs and die a natural death, others are killed by the approach of cold weather, and the rest go into hibernation. Of the immense numbers that go into hibernation but few appear in the spring. This heavy mortality is doubtless due to the varying temperature. A week of warm days appears. The insects, thinking spring has come, desert their protected places and begin feeding. Then, if the night temperature suddenly drops to freezing or below, a great many of them succumb.

The actual dates for the different generations as observed in cages are given in Table VII. Under field conditions it is quite probable that there would be considerable variation from these dates, but they can be considered as an average for the different conditions.

TABLE VII.—*Periods of generations of the three-cornered alfalfa hopper in Arizona in 1912 and 1913*

Generation.	1912	1913
First generation	Feb. 6 to June 10...	Feb. 3 to May 28.
Second generation	June 10 to Aug. 7..	May 28 to Aug. 1.
Third generation	Aug. 7 to Oct. 10...	Aug. 1 to Oct. 1.
Fourth generation	Oct. 10; no eggs.....	Oct. 1; nymphs, in November.

DAMAGE TO ALFALFA

INJURY TO THE PLANT.

The damage to alfalfa and other plants comes as a result of the sucking up of the plant juices for food by the adults and nymphs. The sharp-pointed proboscis-like mouthparts or beak is thrust into the plant and the juice extracted, leaving the plant wilting and often in a dying condition. Both the adults and the young have two methods of feeding. One is a promiscuous puncturing of the stems, while the other is the puncturing in a regular and continuous line which takes the form of a ring or girdle around the stem (Pl. XLIII, fig. 7, *a*). At first it was suspected that this girdling had something to do with egg deposition, since the eggs, being deposited below the girdle which had stopped the circulation of plant fluids, were safe from injury by plant growth. Soon, however, it was noticed that nymphs were more often responsible for the ringing than adults and that girdling from adults had no relation whatever to oviposition.

It is from these girdling punctures that the greatest damage results; for in addition to the loss of plant juices, the stems are weakened, a gall (Pl. XLIII, fig. 7, *b*) usually develops, circulation is cut off from the upper portion of the plant, and a great many of the plants break off, become yellow, and die. It is interesting to note here that the nymphs do more damage than the adults. They seem to be much more hearty

feeders, and, being more sedentary, their feeding is more nearly restricted to a definite area or ring, and with this concentration of work the effect on the plant is more pronounced.

The gall following the girdling of the stem, shown in Plate XLIII, figure 6, *b*, is also quite detrimental to the plant. It is an effort on the part of the plant to mend the injury. There is always a thickened area in the epidermis both above and below the ring, and often this takes peculiar shapes. At one time the swellings will take the shape of globules larger in diameter than the stem itself; at other times a rootlike projection, often half an inch in length, will shoot out; and nearly always, sooner or later, under the pressure of wind or other external influence, the plant will break off and be of little value as food. The more tender stems are always chosen by the insects in preference to the older and more fibrous ones, and thus the maximum of food is found with the least labor. During cool days and cooler weather the feeding is done close to the ground; during warmer weather the species feeds high up on the plant and in the extreme heat of the summer it feeds on the shady side of the stem.

INJURY TO THE CROP

The damage to alfalfa, while not as serious as that caused by some other alfalfa pests, is considerable. To the casual observer it does not appear to be so heavy, chiefly because nothing is seen to be devoured, as in the case of lepidopterous larvæ; and yet, because of the great numbers appearing in alfalfa in the late summer months, farmers have often complained of the hoppers in their fields and have imagined that they were doing damage which in reality was due to larvæ of the yellow alfalfa butterfly (*Eurymus eurytheme*). As has been noted, during the latter part of August and continuing through September that species, as well as a jassid, *Empoasca mali* Le B., attains immense numbers and flies in great swarms before one in an alfalfa field. At this period of the year the alfalfa is fibrous, lacks succulency, and the growth is neither heavy nor thrifty. The hot weather is usually blamed for all this, but the fact is that a considerable percentage of the injury is due to the action of these insects. With dozens of hoppers feeding upon every stem and hundreds upon every plant, all sucking the plant juices, checking plant growth, and girdling many stems, causing them to shrivel and possibly to die or even break off, it is no wonder that the alfalfa looks sickly and is of slow growth during these months. On September 10, 1912, the writer made the following note:

> To-day by motor cycle I went to Chandler, Ariz., to inspect an alfalfa field on Mr. Childs's ranch, which was reported as being "killed off" by insects. Upon reaching the field, I found that the alfalfa was in bad condition. The stems were so scarred from the feeding punctures of *Stictocephala festina* and jassids that they presented a sticky, sickly appearance, and the stems were dry and shrunken so as to be pliable to the touch and not solid and rigid, as they should be. A great many fields to the southeast of Tempe show this damage to a greater or less extent.

From the notes of Mr. T. Scott Wilson I copy the following:

> Tempe, Ariz., September 26, 1913. Stictocephala are very numerous now around Tempe. They are doing a great amount of damage, more than at any time this year. Many alfalfa stalks are completely girdled near the ground and will break off very easily at this ring where the bug has sucked the juice from the stalk. Some have a great many small spots scattered along the stalk where the insects have fed. Other stalks have new branches starting up just below the girdled place, and some are green below this ring and yellow above it. There isn't any doubt (in the writer's mind) that

this is a serious pest to late summer crops. The insects are so thick at present that when a man is walking through alfalfa they fly into his face and swarm ahead of him like bees.

During the early part of September, 1914, several complaints were received from southern Virginia by the United States Department of Agriculture of serious damage to alfalfa by the insect under discussion. One such infestation occurred at the county experiment station at Williamsburg, Va. In this case Mr. R. P. Cocke reported that fully 95 per cent of the plants were seriously affected by the characteristic girdling of the hopper. Specimens of the insect were sent to the Department for identification and found to be the three-cornered alfalfa hopper in its fourth nymphal instar.

DAMAGE TO PLANTS OTHER THAN ALFALFA

In Mississippi Mr. Gibson has found that this hopper does as much damage to cowpeas as it does to alfalfa, or more. He finds that the greatest damage comes when the cowpeas are small, possibly only two or four leaves having developed. In this case, when the plant is girdled it can not so well overcome the damage and usually wilts down immediately. Often as many as 15 nymphs would congregate on one cowpea plant and soon sap its life. One of the serious causes of injury to cowpeas is the oviposition of the females. As has been stated elsewhere in this paper, the eggs are laid in pockets in the stems of the cowpeas, and around these pockets galls often develop. The scars resulting from such action are often so large and so abundant—as many as eight on a single small plant—that the plant is greatly retarded in growth and may break off or die.

Dr. A. W. Morrill, State Entomologist of Arizona, has told the writer that in their work with bean insects they have discovered *Stictocephala festina* in large numbers on beans and probably doing quite a bit of damage. The writer has made no observation of the pest on these plants.

Although a great many other plants are fed upon by this insect, none of them seems to be greatly damaged. While Dr. Oemler, in 1887, reported (4) damage to tomato plants, there seems to be no record since that time of any damage to that crop, and the species has certainly not become of great importance in relation to tomato culture.

NATURAL ENEMIES OF THE ALFALFA HOPPER

During the study of the three-cornered alfalfa hopper as an alfalfa pest it has been shown that it suffers in a remarkably small degree from natural enemies. Prof. T. D. A. Cockerell in 1899 observed (9) a spider, *Argiope transversa* Emerton, feeding on the alfalfa hopper at Phoenix, Ariz. The writer has also noticed remains of the insect in spider webs in alfalfa fields, but these do not exert any remarkable influence in reducing the numbers of the pest. Likewise, the harvester ant (*Pogonomyrmex barbatus* Smith) has been noticed by both the writer and Mr. Gibson carrying individuals of *Stictocephala festina*, but these must have been dead or disabled before capture by the ants. A small red predaceous mite, *Erythraeus* sp., was found feeding upon the eggs, choosing those with the outer end protruding above the plant tissues. Dr. O. C. Bartlett, Assistant State Entomologist of Arizona, informs the writer that in his work with this insect as a bean pest he has reared large numbers

of egg parasites from eggs deposited in the stems of bean plants and expects to publish a report concerning the matter in the near future. The writer, however, has never noted egg parasites issuing from eggs laid in alfalfa stems.

Dr. A. K. Fisher, of the Bureau of Biological Survey, United States Department of Agriculture, informs the writer that they have found stomachs of nighthawks to contain specimens of Stictocephala which these birds must have taken quite early in the evening. Messrs. R. N. and T. Scott Wilson, during September and October, 1913, killed 31 birds that were visiting alfalfa fields, and 10 of these had from one to four adults of *Stictocephala festina* in their crops. Table VIII shows the results of an examination of the stomachs of these birds. The birds were determined by Mr. Frank W. Rogers, State game warden of Arizona.

TABLE VIII.—*Results of the examination of the stomachs of 31 birds killed in alfalfa fields, showing feeding on Stictocephala festina*

Date.	Kind of bird.	Number of stomachs examined.	Number of *Stictocephala* sp. in each stomach.
1913.			
Sept. 12	Killdeer (*Oxyechus vociferus*)	1	1
12	Black phoebe (*Sayornis nigricans*)	1	2
12	Gambel's sparrow (*Zonotrichia leucophrys gambeli*)	6	0
12	Cassin's kingbird (*Tyrannus vociferans*)	1	0
12	Western mourning dove (*Zenaidura macroura marginella*)	1	0
Oct. 24	.....do	2	0
21	Inca dove (*Scardafella inca*)	1	0
21	Western meadow lark (*Sturnella neglecta*)	1	0
24	.....do	2	0
21	Sonoran redwing (*Agelaius phoeniceus sonoriensis*)	3	0 4 0
24	.....do	12	0 3 1 0 0 1 2 1 0 1 0 1

The same investigators killed 19 toads but only found three of these to have *Stictocephala festina* in their stomachs. One stomach contained one nymph and three adults, while two others contained one nymph and one adult, respectively. One would think that toads might feed upon the nymphs to a considerable extent, but the dissections of these 19 stomachs seem to prove the contrary.

PREVENTIVE MEASURES

The great problem is how to control the species. While good may be accomplished by any one of several methods, yet so far no way has been found for entirely controlling the pest. Prof. T. D. A. Cockerell (9) in 1899 suggested that a hopperdozer might be used successfully, but several attempts made during the fall of 1913 by Messrs. R. N. and T. Scott Wilson in which hopperdozers of different forms were used were all unsuccessful. A device with merely the upright canvas back of the oil pan caught only a very small percentage of the alfalfa hoppers. They are so quick and active that they get away without even touching the machine. When a forward projection of cloth was arranged so that the hoppers could not get over the already high back, a few more were taken, but the majority would fly out ahead and to one side, so that a hopperdozer seemed altogether impracticable.

Prof. Osborn (11) suggested timing the removal of the crops so as to destroy the eggs. While it is a certainty that many eggs are destroyed in this way, yet the fact that a large percentage is laid close to the ground and below the point above which they would be removed by the cutting process precludes any possibility of this method being successful.

In several instances fields that were pastured were found to be less infested, but this may have been a coincidence, and at any rate could not be utilized as a method of controlling the pest.

The one practice that will bring about a considerable reduction of the insects is clean methods of farming. When the time comes that each and every farmer is cultivating only as much land as he has means to handle properly—and by the term "handle properly" is meant the tilling of his land in such a way that the maximum returns per acre will be secured—then and then only will insect devastations be reduced to a minimum. The alfalfa hopper can be greatly reduced by just such handling, which must include the eradication of weeds, brush, bunches of wild grass, rubbish, etc., along fences, ditch banks, and other places. The fact that the alfalfa hopper is found during hibernation in places where it is protected from cold and from exposure to its enemies shows that a great many wintering adults may be eliminated by cleaning up such hiding places.

SUMMARY

The three-cornered alfalfa hopper (*Stictocephala festina*) is an insect of economic importance to alfalfa crops in the irrigated valleys of the southwestern United States and to alfalfa and cowpeas in the Southern States.

Injury is due to the sucking of plant juices by both adults and larvæ and the development of a feeding scar which often takes the form of a ring or girdle and which is usually accompanied by a gall formation.

Plants of the legume family constitute the favorite food.

The eggs are deposited in the stems of the food plants, usually back of the sheath leaves or below the surface of the ground. In cowpeas the eggs are deposited in pockets on the stems.

The egg period in Arizona occupies from 12 to 41 days and the five stages of the nymphal period from 22 to 69 days. The average combined length for both periods is about 50 days.

In southern Arizona there are four generations annually and during extremely mild winters the adult insects are active throughout the season. During colder winter the species hibernates in both the egg and adult stages.

The alfalfa hopper is little affected by natural enemies and is only reduced in numbers by the variable winter temperatures. The Sonoran redwing was found to feed upon the species.

The cleaning up of places of hibernation and the eradication of weeds, rubbish, etc., is the only known system that will reduce the numbers of the pest.

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PLATE XLIII

Fig. 1.—The three-cornered alfalfa hopper (*Stictocephala festina*): Adult. *a*, view from side; *b*, view from front. Greatly enlarged. Original.

Fig. 2.—The three-cornered alfalfa hopper: *a*, Nymph in first stage; *b*, egg. Greatly enlarged. Original.

Fig. 3.—The three-cornered alfalfa hopper: Nymph in second stage. Greatly enlarged. Original.

Fig. 4.—The three-cornered alfalfa hopper: Nymph in third stage. Greatly enlarged. Original.

Fig. 5.—The three-cornered alfalfa hopper: Nymph in fourth stage. Greatly enlarged. Original.

Fig. 6.—The three-cornered alfalfa hopper: Nymph in fifth stage. Greatly enlarged. Original.

Fig. 7.—An alfalfa stem showing feeding punctures of the three-cornered alfalfa hopper: *a*, Ring or girdle of punctures around the stem; *b*, gall resulting from girdling. Original.

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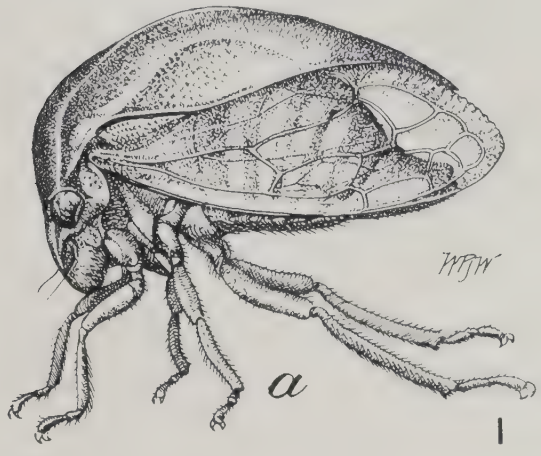

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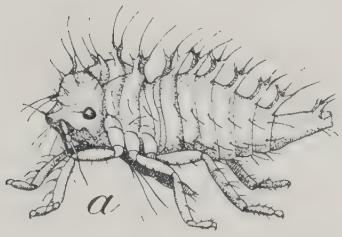

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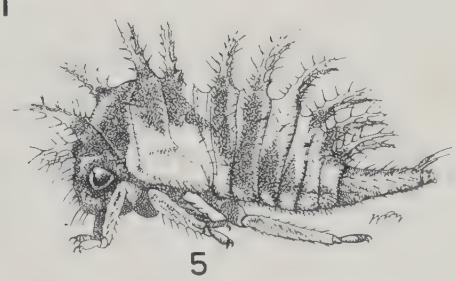

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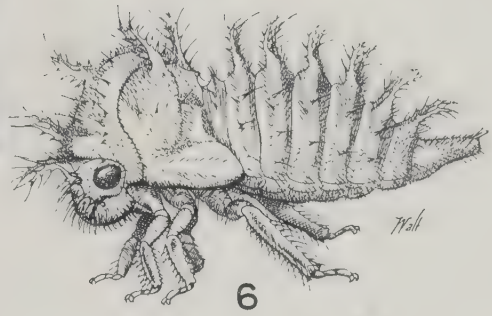

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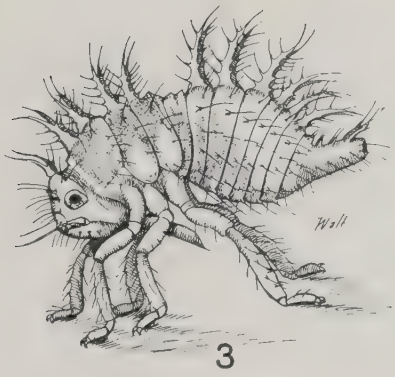

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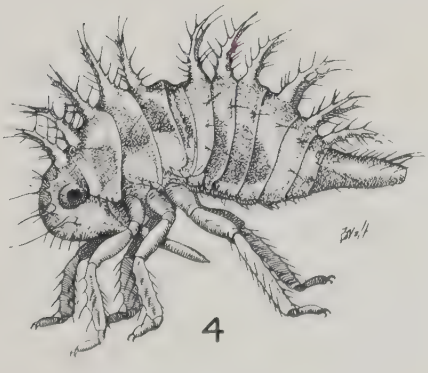

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JOURNAL OF AGRICULTURAL RESEARCH

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LIFE HISTORY OF THE MEDITERRANEAN FRUIT FLY FROM THE STANDPOINT OF PARASITE INTRODUCTION

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INTRODUCTION

The ease with which those parasites of the Mediterranean fruit fly (*Ceratitis capitata* Wied.) that are capable of living several months in glass tubes, if receiving intelligent care, can be introduced from western Africa into Hawaii has been most admirably demonstrated by Dr. F. Silvestri, who was engaged by the Hawaiian Board of Agriculture and Forestry to search western Africa for parasites that might be of value in checking the ravages of the fruit fly in Hawaii.[1] While Dr. Silvestri succeeded in introducing parasites at Honolulu, three species of which (*Galesus silvestrii* Kieffer, *Dirhinus giffardii* Silvestri, and *Opius humilis* Silvestri) have since been reared and liberated in large numbers by the Hawaiian Board of Agriculture and Forestry, he failed to introduce *Tetrastichus giffardii*, which, in his opinion, gives greater promise as a parasite in Hawaii than any of the other species introduced. He ascribes his failure to introduce this parasite to its short life and its habit of ovipositing in either the egg or the young larvæ of the fruit fly. On his trip of exploration, which necessitated many stops and side trips, Dr. Silvestri could not be hampered in his movements by such continuous rearings of the host insect as must be undertaken by one carrying to so great a distance this or other short-lived parasites breeding under similar conditions. During the period of a little more than a year in which the writers have been rearing fruit flies in large numbers they have developed certain extremely simple methods for rearing *Ceratitis capitata*. These result in saving much time and in preventing many failures in connection with the effort to introduce parasites of fruit flies from western Africa into the Hawaiian Islands, as they provide a means of keeping on hand the various stages of the fruit fly for the rearing of new generations of parasites.

[1] For a full account of this expedition, *see* Silvestri, F. Viaggio in Africa per cercare parassiti di mosche dei frutti. 164 p., 69 fig. Portici, 1913. (Bol. Lab. Zool. Gen. e Agr., R. Scuola Sup. Agr. Portici., v. 8, 1914.). For English edition, see Report of an Expedition to Africa in Search of the Natural Enemies of Fruit Flies (Trypaneidae) with Descriptions, Observations, and Biological Notes. 176 p. 24 pl. Honolulu, 1914. (Bul. Hawaii Bd. Agr. and Forestry, no. 3.)

PUPÆ

SECURING MATERIAL

In obtaining a colony of fruit flies the usual method is that of placing infested fruit over sand in some kind of a securely screened container. The writers have found that the easiest method of securing large quantities of pupæ from which to rear adults and pupal parasites is to use any sort of contrivance which will keep the infested fruits free from the sand and at the same time bring the emerging larvæ to a central point, where they may be quickly and easily gathered. If the fruit is allowed to come in contact with the sand, the latter becomes so saturated with the juice of the decaying fruits that it can be freed from the pupæ only with considerable effort and expenditure of time. Plate XLIV, figure 2, represents the first contrivance of this kind used by the writers. It is made of galvanized iron, 36 by 18 inches, with a depth at the lowest point of 24 inches. On the inside, 2 inches from the top, there are narrow supports, which hold in place a tray with handles at both ends and a bottom of galvanized-iron screen with a ½-inch mesh. The larvæ emerging from the fruit instinctively work their way downward and fall through the screen, and are carried thence by gravity and their own movements through the outlet below into the small container. This arrangement works well with very nearly all the host fruits likely to be used as a source of pupæ, such as *Mumsops elangi*, the rose-apple (*Eugenia jambos*), the kamanis (*Terminalia cattapa* and *Calophyllum inophyllum*), and the strawberry guava (*Psidium cattleyanum*). Such fruits as the mango (*Mangifera indica*) can be used, but they yield so much juice that the inside of the frame becomes so wet that the escaping larvæ often pupate without falling through into the container below, and the sand in the latter becomes more or less saturated.

Previous to the adoption of this method of securing pupæ, there was in general use, both by the Hawaiian Board of Agriculture and Forestry and by the writers, a shallow box about 14 by 12 by 3 inches, ordinarily used by florists. The bottom of this box was covered to a varying depth with sifted sand, on which were placed the infested fruits. During the height of the season of 1913 from five to eight men were employed by the Territorial and Federal authorities in daily removing infested fruits from one box to another containing fresh sand, and in sifting for the pupæ the sand over which the fruit had lain for 24 hours. Besides requiring much time, the prolonged sifting necessary to free the pupæ injured many of them. The writers are now using a frame 6 by 3 feet and 3 feet in depth similar to that shown in Plate XLIV, figure 2. The use of a sufficient number of cheap wooden frames covered with tin well painted will make it possible for one man in several hours to do the daily work formerly requiring six to eight men.

An adaptation of the old box system has been found useful when it has been desirable to keep separate the pupæ from small lots of fruit gathered

from various localitites. One box with a screen bottom of a sufficiently large mesh contains the fruit and is placed over a box of the same size containing sand. By this method the fruit does not need to be handled daily, and the sand below is kept so dry that the larvæ falling through into it are easily sifted (Pl. XLIV, fig. 1).

EMERGENCE OF LARVÆ AND PUPATION

The larvæ leave the fruit in largest numbers at or just after daybreak. Thus, on the 6th of July, 16,624 larvæ emerged between 4 and 9 a. m., as compared with 57 between 9 a. m. and 11 p. m. Siftings made at 6, 7, 8, 9, and 10 a. m., on July 7, yielded 1,006, 448, 171, 95, and 14 larvæ, respectively, during these hourly intervals. On July 8, at 5.20, 6, 7, 8, 9, and 10 a. m., 152, 978, 369, 72, 31, and 14 larvæ, respectively, emerged, as compared with 7 larvæ during the rest of the day. The mean temperature for the period of larval emergence during which these observations were made ranged from 73° to 74° F. Nearly all puparia are formed in from one to two hours during warm weather.

LENGTH OF PUPAL STAGE

From the data included in Table I it will be seen that the minimum length of the pupal stage is 6 days when the mean temperature ranges from about 76° to 79° F. During the warmest Honolulu weather the larger proportion of any lot of pupæ requires from 9 to 11 days before yielding adults. This period may be increased to at least 19 days when the daily means drop to about 69° to 71°.

TABLE I.—*Duration of the pupal stage of the Mediterranean fruit fly*

Date of pupation.	Date of emergence.	Number of adults emerging.	Pupal stage.	Mean temperature.	Date of pupation.	Date of emergence.	Number of adults emerging.	Pupal stage.	Mean temperature.
			Days.	° *F.*				*Days.*	° *F.*
Jan. 16	Feb. 1	1	16	69.6	Apr. 17	Apr. 29	43	12	69.6
Jan. 18	Feb. 3	1	16	70.0	Do....	Apr. 30	1	13	72.7
Jan. 19	Feb. 4	38	16	70.0	Apr. 20	May 1	24	11	73.2
Jan. 20	...do.....	18	15	70.0	Do....	May 2	10	12	73.2
Jan. 23	Feb. 5	10	13	70.3	Apr. 22	...do.....	1	10	73.4
Jan. 26	Feb. 10	16	15	71.2	Do....	May 3	11	11	73.5
Feb. 13	Feb. 27	130	14	70.8	Apr. 23	May 5	6	12	73.7
Feb. 14	Feb. 28	250	14	70.8	Apr. 24	...do.....	9	11	73.9
Feb. 16	Mar. 2	300	14	71.2	Apr. 25	...do.....	1	10	73.7
Feb. 18	Mar. 3	300	14	70.3	Do....	May 6	10	11	73.7
Feb. 27	Mar. 13	7	14	70.4	June 4	June 13	1	9	76.1
Do.....	Mar. 14	40	15	70.3	Do....	June 14	3	10	76.1
Do.....	Mar. 15	15	16	70.4	Do....	June 15	73	11	76.2
Do.....	Mar. 17	17	18	70.9	June 8	June 16	2	8	76.2
Do.....	Mar. 18	3	19	70.9	Do....	June 17	15	9	76.2
Feb. 28	Mar. 15	88	15	70.5	Do....	June 18	28	10	76.1
Do.....	Mar. 16	56	16	70.9	Do....	June 19	53	11	76.1
Do.....	Mar. 17	14	17	71.0	Do....	June 20	33	12	76.1
Mar. 1	Mar. 16	29	15	70.2	Do....	June 21	5	13	76.1
Do.....	Mar. 17	9	16	71.1	June 10	June 16	5	6	76.4
Mar. 14	Mar. 26	1	12	73.9	Do....	June 19	14	9	76.3
Do.....	Mar. 27	1	13	73.7	Do....	June 20	101	10	76.3
Apr. 11	Apr. 22	4	11	71.2	Do....	June 21	160	11	76.2
Do.....	Apr. 23	8	12	71.4	Do....	June 22	7	12	76.1
Do.....	Apr. 24	3	13	71.6	Do....	June 23	3	13	76.3
Apr. 13	...do.....	6	11	72.0	July 13	July 20	1	7	79.2
Do.....	Apr. 25	4	12	72.9	Do....	July 21	3	8	79.2
Apr. 15	Apr. 26	2	11	72.4	Do....	July 22	39	9	79.2
Do.....	Apr. 27	3	12	72.5	Do....	July 23	43	10	79.1
Apr. 17	Apr. 28	15	11	72.6	Do....	July 24	3	11	79.0

It was found that in Bermuda, when the monthly means range from 62.5° to 64.8° F., the pupal stage was lengthened to about 31 days under normal conditions. The writers have found that the Mediterranean fruit fly can pass from egg to adult if kept in the dark in cold storage at 56° to 57° and that at this temperature practically all pupæ yield adults from 37 to 41 days after pupation. Pupæ placed in cold storage in the light at a temperature varying between 58° and 62° were apparently unaffected by the cold, except that the length of the stage was increased to from 29 to 31 days for pupæ which were about 3 hours old when placed in cold storage. In carrying pupæ from place to place for rearing purposes a temperature of less than 56° to 60° is not advised, as great mortality occurs. Thus, from about 300 pupæ 1 day old placed in cold storage at about 50° on June 2 and removed to a normal summer temperature at Honolulu on July 22 only 8 adults emerged during the period from July 24 to 26.

ADULTS

The adults of the Mediterranean fruit fly emerge in largest numbers early in the morning during warm weather and more scatteringly during cool weather.

CARE OF ADULTS

As adults die in greatest numbers within 48 hours after emergence, or within 72 hours at the longest, if food is not given them, those required for future observations must be transferred to a place where they may be fed and cared for daily. In this work the writers have found glass jars 9 by 12½ inches covered with cheesecloth very convenient. Such jars will hold from 200 to 300 flies in good condition. Fruit juices of almost any sort are eagerly eaten. Water slightly sweetened with pineapple (*Ananas ananas*) syrup was used with good results by the writers for many months, but was replaced later by a mixture of water and finely divided parts of papaya (*Carica papaya*). When fed with such diluted food, adults thrive best on two feedings a day, one in the morning and one late in the afternoon. The food may be applied in finely divided drops to the sides of the jar by flirting the mixture forcibly against the cheesecloth covering by means of a snapping movement of the thumb and forefinger. The adults feed greedily and soon become distended. In this condition many fall and rest upon the bottom of the jar; hence, the less food falling on this portion of the jar the fewer will be the deaths resulting from entanglement in it. If the flies are not required for oviposition, they can be kept alive far more easily by suspending within the jar a juicy fruit upon which they may feed. One mango, the skin of which had been broken in numerous places, served to keep alive from 100 to 200 flies for one week. Feeding by flirting mixtures through the cloth covering causes the sides of the jar to become soiled quickly and necessitates

the changing of adults to clean jars every two or three days. When fed with suspended fruit, the flies require no changing for at least two weeks. Mortality among the flies increases rapidly in badly soiled jars.

LENGTH OF LIFE

Well-fed Mediterranean fruit flies have been kept alive in these jars more than five months. One fly that emerged on December 31, 1913, lived until May 11, 1914, or 131 days. Other flies, which emerged on February 28, 1914, are alive at the date of this writing—August 1. Usually about 50 per cent of the flies may be expected to die during the first two months after emergence. When the monthly mean temperature averages about 76° to 79° F., comparatively few flies live to be more than 3 months old. When kept at a temperature ranging from 58° to 63°, the writers believe from accumulating data, that especially strong adults will live to be more than 6 months old.

SEXUAL MATURITY

Neither male nor female flies are sexually mature when they emerge from the pupa. Males show sexual activity often four days after emergence, and copulation has been observed five days after emergence. When the daily mean temperature averages from 76° to 78° F., the larger percentage of females is ready to mate from six to eight days after eclosion. Adults that emerged on May 23 and 24 and were placed on May 25 in the light in cold storage at 61° to 64° were not observed to mate until June 5, when 14 days old. Copulation may occur at any time throughout the day.

OVIPOSITION

Oviposition may take place in Hawaii as early as 5 days after emergence during very warm weather, but not for about 10 days when the temperature ranges between 68° and 72° F. At mean temperatures above 74° various lots of adults will yield large numbers of eggs from 7 to 8 days after emergence. Adults oviposit best at temperatures varying from 70° upward, but have been observed depositing eggs at 65° to 67°, and in cold storage in the light at about 62°.

It is impossible at this writing (August 1, 1914) to state the full capacity for egg deposition possessed by females of this species. The number of eggs found at any time in the reproductive organs is no indication of the total number of eggs an individual female is capable of depositing, for new eggs are being formed continually throughout life. The data in Table II show that during the first 18 weeks of her life one adult deposited 499 eggs and was still in a thrifty condition. Two other females during the same time deposited 416 and 336 eggs, respectively. A fourth female living but 80 days deposited 312 eggs. Usually females die soon after

they cease to oviposit. Fly No. 9 in Table II is an exception, as she deposited but 3 eggs in one puncture during her life of 68 days and lived without ovipositing for 35 days before she died. The data in Table II give the capacity for oviposition possessed by females up to 18 weeks of age. Those in Table III show that this capacity is fairly well maintained by certain females at least during the fifth month after emergence. Thus fly No. 5 of Table II deposited on an average 4.5 eggs per day after she began ovipositing, while fly No. 1 of Table III deposited an average of 4.6 eggs per day for the first 24 days of the fifth month of her life.

TABLE II.—*Daily rate of oviposition of the Mediterranean fruit fly. Females emerged on April 4, 1914, and were placed with fruit on April 14, 1914*

Date of oviposition.[a]	Number of eggs deposited.								
	Fly No. 1.	Fly No. 2.	Fly No. 3.	Fly No. 4.	Fly No. 5.	Fly No. 6.	Fly No. 7.	Fly No. 8.	Fly No. 9.
Apr. 16	3	0	0	0	0	0	0	0	0
17 to 20	14	7	11	24	19	14	0	0	0
20 to 22	0	0	0	0	0	0	5	0	0
22 to 25	0	0	7	0	0	0	0	2	0
25 to 27	0	7	13	0	13	0	14	0	0
27 to 29	11	8	6	0	16	15	0	20	0
29 to 30	0	0	0	0	17	16	23	0	0
May 1 to 3	0	0	0	3	25	19	19	13	0
3 to 5	25	0	0	0	12	0	0	0	3
6	2	0	0	0	0	0	9	0	0
7	8	0	0	0	19	12	2	8	0
8	9	0	0	0	9	3	1	0	0
9	9	0	0	0	7	7	4	3	0
10	0	0	0	2	0	6	2	0	0
11	17	0	0	0	7	3	0	0	0
12	4	4	0	0	10	4	8	0	0
13	14	0	0	0	7	8	11	0	0
14	8	4	0	0	10	2	9	0	0
15	8	0	0	5	5	6	6	1	0
16	5	0	0	0	11	6	8	14	0
17	8	0	0	0	0	4	3	0	0
18	2	0	0	13	3	3	3	8	0
19	3	4	0	0	5	0	3	9	0
20	2	(b)	0	0	6	9	3	11	0
21	0	.....	0	0	2	2	9	8	0
22	5	.....	0	0	5	0	1	9	0
23	10	.....	20	4	6	10	4	4	0
24	4	.....	2	18	3	1	4	7	0
25	5	.....	19	4	2	3	3	3	0
26	0	.....	2	9	6	2	0	3	0
27	0	.....	6	15	9	0	5	8	0
28	0	.....	(c)	3	3	5	3	4	0
29	9	.....	.....	9	3	14	7	7	0
30	1	.....	.....	12	0	8	10	4	0
31	0	.....	.....	9	6	13	21	8	0
June 1	0	.....	.....	8	3	10	5	5	0
2	0	.....	.....	13	5	6	14	6	0
3	0	.....	.....	9	12	4	7	2	0

[a] Dates on which none of the flies oviposited are omitted from the table.
[b] Died on this date.
[c] Escaped on this date.

TABLE II.—*Daily rate of oviposition of the Mediterranean fruit fly. Females emerged on April 4, 1914, and were placed with fruit on April 14, 1914*—Continued

Date of oviposition.	Number of eggs deposited.								
	Fly No. 1.	Fly No. 2.	Fly No. 3.	Fly No. 4.	Fly No. 5.	Fly No. 6.	Fly No. 7.	Fly No. 8.	Fly No. 9.
June 4	5	.....	.....	3	3	4	3	9	0
5	0	.....	.....	7	8	0	6	5	0
6	0	.....	.....	4	6	0	4	3	0
7	0	.....	.....	18	11	15	15	12	0
8	0	.....	.....	0	0	1	0	1	0
9	0	.....	.....	15	7	8	12	9	0
10	0	.....	.....	6	5	11	1	2	(a)
11	0	.....	.....	12	7	11	9	6	.....
12	(a)	.....	.....	0	6	3	3	6	.....
13	.....	.....	.....	7	2	4	7	0	.....
14	.....	.....	.....	4	2	7	1	8	.....
15	.....	.....	.....	0	7	2	7	2	.....
16	.....	.....	.....	0	9	4	5	3	.....
17	.....	.....	.....	0	6	5	0	2	.....
18	.....	.....	.....	0	0	0	0	3	.....
19	.....	.....	.....	0	6	3	3	8	.....
20	.....	.....	.....	4	4	12	0	4	.....
21	.....	.....	.....	10	10	7	0	9	.....
22	.....	.....	.....	4	0	0	0	5	.....
23	.....	.....	.....	10	2	(a)	9	0	.....
24	.....	.....	.....	0	9	.....	12	12	.....
25	.....	.....	.....	0	7	.....	3	3	.....
26	.....	.....	.....	0	9	.....	0	2	.....
27	.....	.....	.....	5	6	.....	7	4	.....
28	.....	.....	.....	16	14	.....	12	4	.....
29	.....	.....	.....	6	5	.....	0	2	.....
30	.....	.....	.....	0	2	.....	6	2	.....
July 1	.....	.....	.....	3	7	.....	2	8	.....
2	.....	.....	.....	10	0	.....	7	1	.....
3	.....	.....	.....	3	0	.....	3	1	.....
4	.....	.....	.....	7	4	.....	8	6	.....
5	.....	.....	.....	0	6	.....	2	3	.....
6	.....	.....	.....	0	3	.....	4	0	.....
7	.....	.....	.....	0	2	.....	0	0	.....
8	.....	.....	.....	(a)	3	.....	9	2	.....
9	.....	.....	.....	.....	6	.....	3	4	.....
10	.....	.....	.....	.....	2	.....	3	4	.....
11	.....	.....	.....	.....	0	.....	8	2	.....
12	.....	.....	.....	.....	9	.....	4	0	.....
13	.....	.....	.....	.....	2	.....	0	0	.....
14	.....	.....	.....	.....	5	.....	3	5	.....
15	.....	.....	.....	.....	2	.....	0	3	.....
17	.....	.....	.....	.....	4	.....	0	0	.....
19	.....	.....	.....	.....	0	.....	0	4	.....
20	.....	.....	.....	.....	0	.....	2	0	.....
22	.....	.....	.....	.....	14	.....	0	0	.....
23	.....	.....	.....	.....	0	.....	3	0	.....
24	.....	.....	.....	.....	11	.....	4	0	.....
Total	191	34	86	314	[b] 499	312	[b] 416	[b] 336	3

[a] Died on this date.
[b] Aug. 1, 1914: These females are still alive and promise to oviposit for some time yet.

TABLE III.—*Daily rate of oviposition of the Mediterranean fruit fly. Females emerged on February 28, 1914; hence, were 4 months old on June 28, 1914*

Date of oviposition.[a]	Number of eggs deposited.					
	Fly No. 1.	Fly No. 2.	Fly No. 3.	Fly No. 4.	Fly No. 5.	Fly No. 6.
July 1	0	0	0	0	6	0
2	9	0	0	0	2	0
3	0	0	0	0	4	0
4	10	4	0	0	0	6
5	4	0	0	0	0	0
6	5	6	0	4	0	0
7	0	0	0	0	0	12
8	10	6	0	0	0	6
9	0	4	0	9	0	13
10	15	6	0	3	0	2
11	5	2	0	0	0	4
13	12	7	(b)	5	0	0
14	2	0	.....	5	0	(b)
15	7	9	.....	0	0	.....
16	0	8	.....	0	0	.....
17	13	3	.....	11	0	.....
18	5	0	.....	4	0	.....
19	6	2	.....	0	0	.....
20	0	5	.....	4	0	.....
21	5	2	.....	6	0	.....
22	0	8	.....	7	0	.....
23	0	2	.....	0	0	.....
24	2	6	.....	13	(b)	.....
Total	[c] 110	[c] 80	0	[c] 71	12	43

[a] Dates on which none of the flies oviposited are omitted from the table.
[b] Died on this date.
[c] Aug. 1, 1914: These females are still alive and give promise of sexual activity for some time to come.

Oviposition experiments are still in progress; hence, no estimate can be made of the maximum egg-laying capacity of the female *Ceratitis capitata*. It is evident, however, from the data in Tables II and III that the females lay small batches of eggs quite regularly throughout life.

DIFFERENCES IN HABIT BETWEEN THE ADULT MEDITERRANEAN FRUIT FLY AND THE ADULT MELON FLY

Those desiring to rear the melon fly (*Bactrocera cucurbitae* Coq.) or parasites of its eggs and young larvæ will find marked differences in habit between this and *Ceratitis capitata*. These same differences will probably be found to occur between species of Dacus and Ceratitis. There is very little difference found by the writers in the egg, larval, and pupal stages. The adults of the melon fly are far more hardy than adults of *C. capitata*. The writers have on hand many adults 6 months old which give every promise of living indefinitely, as very few have died during the last few months and those living are as active as when newly emerged. The adult melon fly exhibits no sexual activity for such a long period

after emergence that one is likely to be discouraged in obtaining eggs. While the sexes of the Mediterranean fruit fly are sexually active throughout the day, the melon flies become active only at sunset. From sunset until dark copulation occurs and lasts in many instances until daybreak, inasmuch as numerous pairs have been observed in coition at midnight and at dawn, when all flies are very quiet. Adults issuing from pupæ on May 24 did not mate until June 13, or 20 days after emergence, although they were observed every evening. The majority of females in this lot did not mate until 25 days old. The daily mean temperatures for the period from May 24 to June 13 averaged 75.5° F.

The female *Bactrocera cucurbitae* is more irregular in her habits of oviposition. As shown by the data in Table IV, she lays more consistently a large number of eggs at one time.

TABLE IV.—*Daily rate of oviposition of the melon fly (Bactrocera cucurbitae). Emerged on May 25 and placed separately with fruit on June 25, 1914*[a]

Date of oviposition.[b]	Number of eggs deposited.						
	Fly No. 1.	Fly No. 2.	Fly No. 3.	Fly No. 4.	Fly No. 5.	Fly No. 6.	Fly No. 7.
July 10	0	0	0	0	0	23	0
11	0	0	13	0	17	0	0
15	14	0	0	0	0	12	0
17	0	0	9	0	14	0	0
18	19	0	0	0	0	0	0
19	0	0	0	0	0	0	19
21	0	0	0	0	0	6	0
22	13	0	0	0	0	0	0
23	0	0	0	0	10	0	0
24	0	0	0	3	0	0	0
26	0	0	0	0	0	23	0
27	29	0	0	0	0	0	0

[a] These 7 females were all alive on July 27.
[b] Dates on which none of the flies oviposited are omitted from the table.

The data in Table IV were secured from young females during the early period of sexual activity. Other data on file show that females over 5 months old deposit quite as freely.

EGGS

Eggs may be obtained most easily for experimental work by suspending fruit on a string in a jar containing adults, after the latter have begun to mate. In Plate XLV, figure 1, is illustrated this simple method of obtaining eggs during a known period. If the epidermis of the fruit is shaved off in several places oviposition will be made easier. The removing of eggs either from the body of the female or from the sides of the containing jar, as practiced by several workers, has not given good results.

If it is desired to keep constant watch over eggs they may be dissected easily from the egg cavity and spread upon a section cut from any firm

leaf. The leaf should then be inserted into a small vial, an absorbent-cotton plug added to force the leaf well toward the bottom, and the vial with contents inverted and partially submerged in a jar of water. It has been found by checks that eggs, when handled in this manner, develop normally unless injured in the transfer. An ordinary moist chamber does not seem to serve the purpose so well.

TABLE V.—*Duration of the egg stage of the Mediterranean fruit fly*

Number of eggs under observation.	Eggs deposited.	Eggs hatched.	Average mean temperature.
			°F.
88	Jan. 21–22, 4 p. m. to 10 a. m.	Jan. 26, 6 a. m. to 3 p. m.	68. 7
350	Mar. 9	Mar. 12–13, 4.30 p. m. to 8 a. m.	70. 2
264	Mar. 22–23, 2 p. m. to 9 a. m.	Mar. 26, a. m.	71. 0
135	Do.	Mar. 27, a. m.	71. 0
236	Do.	Mar. 28–29, 8 a. m. to 6 a. m.	71. 3
12	Do.	Mar. 29–30, 6 a. m. to 10 a. m.	71. 0
102	Mar. 27, 9 a. m. to 1 p. m.	Mar. 30, a. m.	71. 0
695	Do.	Mar. 30, a. m., to Mar. 31, a. m.	71. 0
28	Do.	Mar. 31–Apr. 1, 9 a. m. to 8 a. m.	71. 0
3	Do.	Apr. 1–2, 9 a. m. to 8 a. m.	71. 0
50	Mar. 27, a. m.	Mar. 31, a. m.	71. 0
102	May 19, 3 p. m. to 6 p. m.	May 22, 7 a. m. to 10 a. m.	76. 0
176	May 12–13, 3 p. m. to 12 m.	May 15, a. m.	75. 0
243	Do.	May 15–16, 2 p. m. to 8 a. m.	75. 0
13	June 17–18, 4 p. m. to 8 a. m.	June 20, 11 a. m.	77. 0
2	Do.	June 19–20, 6 p. m. to 8 a. m.	77. 3
44	June 18, 1.30 p.m. to 3.30 p. m.	June 20–21, 6 p. m. to 8 a. m.	77. 0
90	June 19, 10 a. m. to 1 p. m.	June 21–22, 6 p. m. to 8 a. m.	76. 6
72	June 19–20, 4 p.m. to 8.30 a.m.	June 21–22, 6 p. m. to 9 a. m.	77. 0
77	June 20, 9 a. m. to 4 p. m.	June 22–23, 6 p. m. to 7.30 a. m.	77. 0
60	June 23, 10 a. m. to 4 p. m.	June 25–26, 6 p. m. to 6 a. m.	76. 8
12	Do.	June 26, 7 a. m. to 11.45 a. m.	76. 8
63	June 24, 1.30 p.m. to 4.30 p.m.	June 26–27, 4.30 p. m. to 6 a. m.	77. 0
24	July 1, 1.30 p. m. to 5 p. m.	July 3–4, 5 p. m. to 6.30 a. m.	77. 0
134	July 15, 3.30 p. m. to 4.30 p.m.	July 17, 4.30 p. m. to 6 p. m.	78. 9
128	Do.	July 17, 6 p. m. to 8 p. m.	78. 9
20	Do.	July 17, 9 p. m. to 10 p. m.	78. 9
12	Do.	July 18, 10 a. m to 4 p. m.	78. 3
74	July 15–16, 12 m. to 5 p. m.	July 18	80. 0
18	Do.	July 19, a. m.	79. 8
2	Do.	July 19, 9 a. m. to 1 p. m.	79. 8
2	Do.	July 20, p. m.	79. 5
101	Nov. 13–14, 4 p. m. to 9 a. m.	Nov. 16, 2 a. m. to 6 a. m.	75. 5
10	Do.	Nov. 16, 6 a. m. to 11 a. m.	75. 5
3,442			

Plate XLV, figure 2, is reproduced from a photograph of an apple that had been hung in a jar with flies for one day. Each dark spot represents a puncture. The entire apple was estimated to contain over 2,000 eggs. As the females live over long periods and oviposit freely throughout life eggs may easily be obtained daily for parasitic work while experimenters are en route from one country to another. Apples are

probably the most satisfactory fruit for egg deposition during a voyage, as they may be had at almost all points and they keep for a considerable length of time. While the female shows decided preference for certain fruits, she will oviposit, when forced, in almost any fruit if oviposition is not prevented by physical conditions. Fruit in a hard and semiripe condition is better for oviposition than fully ripe fruit, as the latter is likely to be more juicy, and very juicy fruits often cause a high mortality among eggs and young larvæ.

During very warm weather eggs hatch in about two days. It will be seen, however, from the data in Table V that the length of the egg stage is considerably increased by lower temperatures.

At a mean temperature of 78.9° F. 134 eggs hatched between 49 and 50 hours after being deposited, although 12 eggs deposited at the same time did not hatch until from 66 to 72 hours. At a mean temperature of 71° F. 695 eggs hatched within 72 hours, while 3 hatched in from 120 to 144 hours, or about 6 days after deposition. Eight eggs hatched between 4 and 4½ days after deposition at a mean temperature of 68.7°. At 59° to 62° eggs hatched in cold storage in from 5 to 7 days, and at 54° to 57° in from 7 to 14 days after deposition.

LARVÆ

The larvæ pass through three instars, which may be readily distinguished. Of chief interest in connection with this paper is the length of larval life. The data in Table VI show that this may be as short as 5 or 6 days when the mean temperatures average about 77° F. One larva at this temperature required 14 days to become full grown.

The character of the fruit often influences the length of the larval stage. In citrous fruits, especially in limes and lemons, it appears to be longer. Thus larvæ require 14 to 26 days to reach maturity in a ripe lemon, as compared with 10 to 15 days in a green peach. Citrous fruits, however, are not desirable for rearing work with either flies or their egg parasites. For successful rearing work, where it is desired to prolong the length of larval life by slightly lowered temperatures, adults should not be permitted to lay more than 50 to 150 eggs in such fruits as the apple. The feeding of larvæ in overinfested fruits brings about such a rapid decay that few become well grown. At 56° to 57° F. larvæ have become full grown and emerged from slightly infested apples in a refrigerator over a period ranging from 36 to 53 days after hatching.

TABLE VI.—*Duration of the larval stage of the Mediterranean fruit fly*

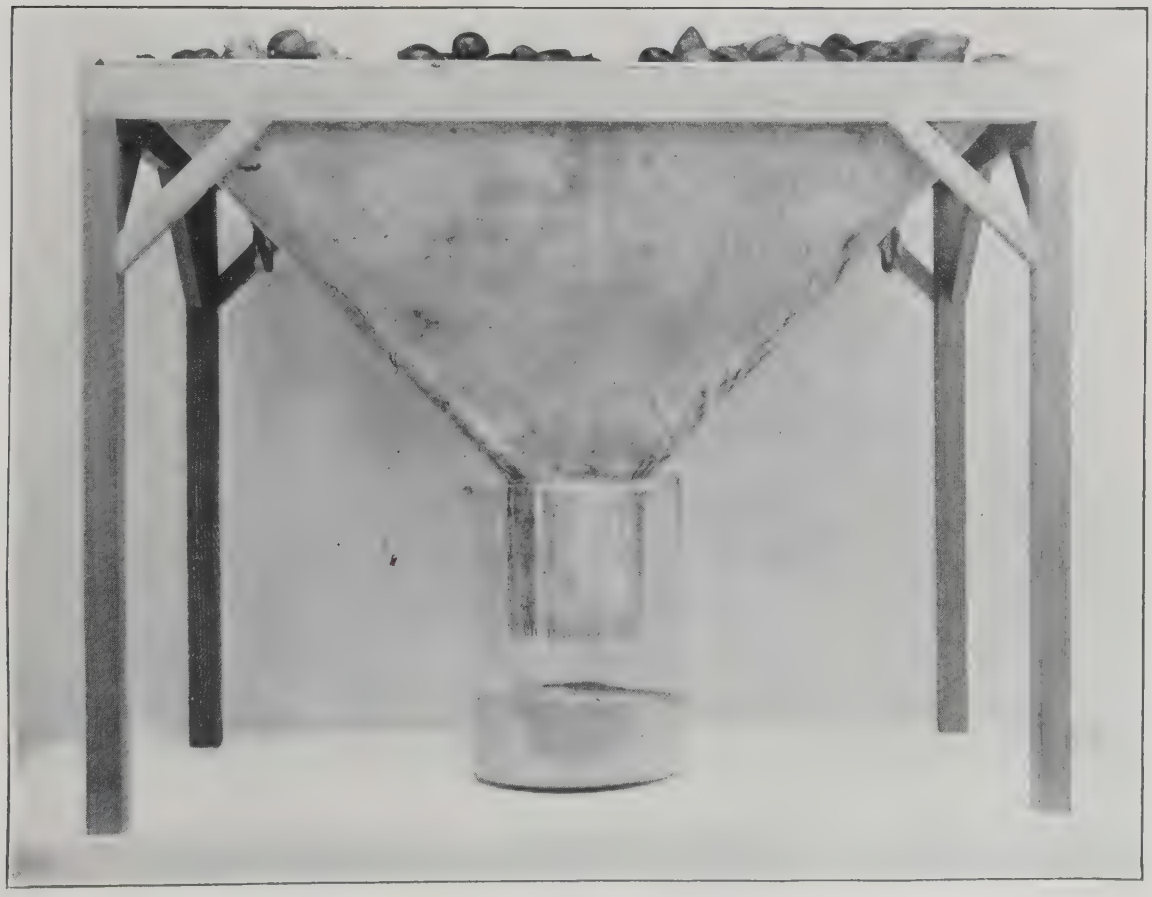
Number of specimens under observation.	Approximate period of development.	Host fruit.	Instar 1.	Instar 2.	Instar 3.	Larval stage.	Mean temperature for period of development.
			Hrs.	*Hrs.*	*Hrs.*	*Days.*	°*F.*
1	June 12 to 18	Papaya	38	36	48	5.1	77.6
2	Do	do	36	48	48	5.5	77.6
1	Do	do	36	48	48	5.5	77.6
1	Do	do	48	48	48	6.0	77.6
1	June 19 to 25	do	26	30	72	5.3	76.4
1	Do	do	48	24	72	6.0	76.4
12	June 19 to 26	do	48	24	96	7.0	76.6
1	June 19 to 27	do	48	30	96	7.2	76.6
1	June 19 to July 1	do	48	24	216	12.0	77.0
1	June 19 to July 3	do	48	27.5	264	14+	77.1
2	June 22 to July 2	Green peach	.....	.....	.....	10	77.2
7	June 22 to July 3	do	.....	.....	.....	11	77.2
6	June 26 to July 6	Hard peach	.....	.....	.....	9.5	77.4
4	June 26 to July 2	Ripe peach	.....	.....	.....	6	77.8
3	June 26 to July 3	do	.....	.....	.....	7	77.7
18	Mar. 31 to Apr. 10	Green peach	.....	.....	.....	10	69.6
12	Mar. 31 to Apr. 11	do	.....	.....	.....	11	69.8
3	Mar. 31 to Apr. 12	do	.....	.....	.....	12	70.0
1	Mar. 31 to Apr. 13	do	.....	.....	.....	13	70.3
1	Mar. 31 to Apr. 15	do	.....	.....	.....	15	71.0
12	Mar. 13 to 27	California lemon	.....	.....	.....	14	70.2
17	Mar. 13 to 28	do	.....	.....	.....	15	70.3
14	Mar. 13 to 29	do	.....	.....	.....	16	70.3
20	Mar. 13 to 30	do	.....	.....	.....	17	70.4
8	Mar. 13 to 31	do	.....	.....	.....	18	70.4
3	Mar. 13 to Apr. 1	do	.....	.....	.....	19	70.5
3	Mar. 13 to Apr. 2	do	.....	.....	.....	20	70.5
2	Mar. 13 to Apr. 3	do	.....	.....	.....	21	70.4
1	Mar. 13 to Apr. 4	do	.....	.....	.....	22	70.4
1	Mar. 13 to Apr. 8	do	.....	.....	.....	26	70.4

PLATE XLIV

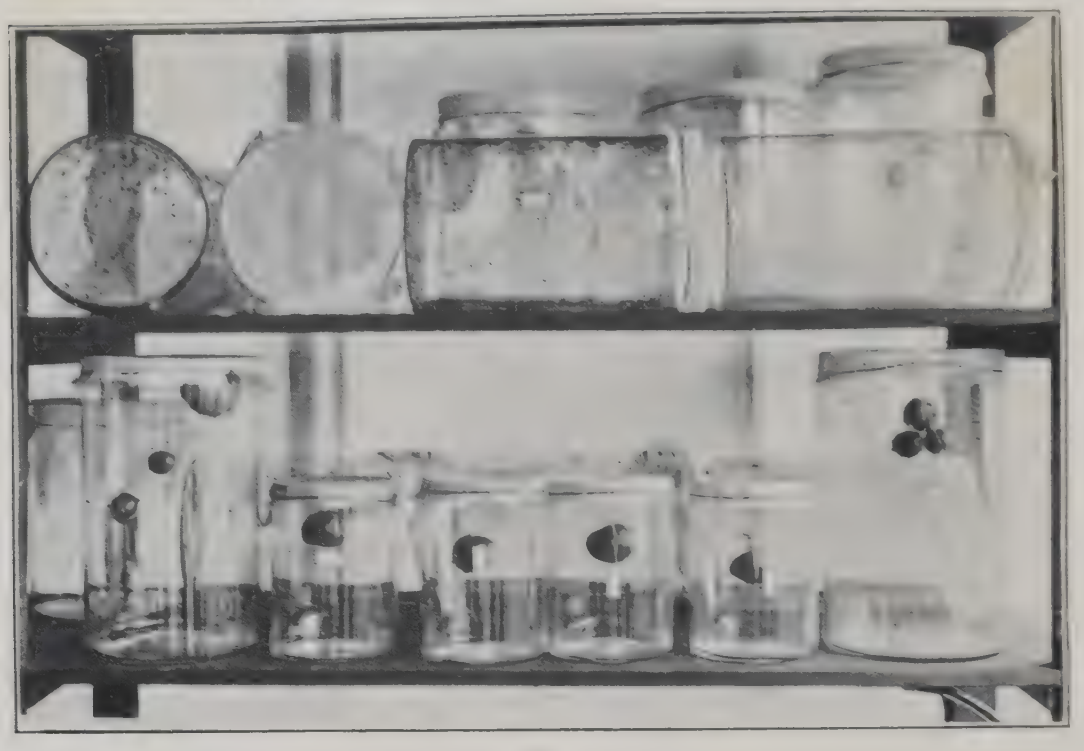
Fig. 1.—Wooden boxes, 14 by 12 by 3 inches in size, used in obtaining pupæ of fruit flies. The upper box, with a coarse screen bottom, contains the infested fruit; from which the larvæ drop through the screen into the lower box containing dry sand. Original.

Fig. 2.—Contrivance used for keeping the infested fruit free from the sand and bringing the emerging larvæ to a central container where they may be gathered quickly. Original.

1



2



1

2

PLATE XLV

Fig. 1.—Method of keeping adult fruit flies alive over long periods. The jars in the lower row contain suspended fruits in which the females readily oviposit. Original.

Fig. 2.—An apple after having been suspended for one day in a jar containing Mediterranean fruit flies. Each dark spot represents a puncture containing from 1 to 30 eggs. The apple is too heavily infested for practical work in rearing parasites. Original.

RELATION OF SIMULTANEOUS OVULATION TO THE PRODUCTION OF DOUBLE-YOLKED EGGS[1]

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INTRODUCTION

In an earlier paper[2] it was shown that double-yolked eggs differ greatly in the number of the normal egg envelopes common to the two yolks. The explanation was then offered that two eggs may come together at any level of the oviduct. If the union is anterior to the isthmus ring, a double-yolked egg results. It was also pointed out that while some of the doubling of eggs is no doubt due to the delay or the backward movement of the first egg, nevertheless an unusually rapid succession of ovulations is necessary to account for the occurrence of double-yolked eggs within a clutch.

The purpose of this paper is, first, to present further data regarding the structural variations in double-yolked eggs and the relation of these variations to the functional divisions of the oviduct; and, second, to record observations bearing upon the relation between simultaneous ovulations and double-yolked egg production.

CLASSIFICATION OF DOUBLE-YOLKED EGGS BASED ON THE NUMBER OF COMMON EGG ENVELOPES

In the earlier paper[3] it was shown that yolks with separate vitelline membranes may have a complete set of common egg envelopes, including common chalazal membranes—"chalaziferous layers" (Pls. XLVI, fig. 3, and XLVII, fig. 1); or they may have separate chalazal membranes, all their other envelopes being common (Pl. XLVII, fig. 2); or they may have some separate and some common thick albumen layers (Pl. XLVIII, fig. 1); or finally they may have entirely separate thick albumen layers but common egg membrane and shell (Pl. XLVIII, fig. 2). A large series of double-yolked eggs shows every possible stage, from cases where the two yolks are flattened together so tightly within the common chalazal membrane that they resemble a single large yolk to eggs in which the doubleness is visible externally by a depressed ring around the shell (Pl. XLIX, fig. 1). In such cases there is often a thin fold of membrane

[1] Studies on the Physiology of Reproduction in the Domestic Fowl.—XI. This paper is the eleventh in a series published in various biological journals and agricultural experiment station bulletins.

Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 75.

[2] Curtis, M. R. Studies on the physiology of reproduction in the domestic fowl.—VI. Double- and triple-yolked eggs. *In* Biol. Bul., v. 26, no. 2, p. 55–83. 1914.

[3] Curtis, M. R. Op. cit.

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projecting into the albumen at the deepest part of the furrow (Pl. XLIX, fig. 2). Although the eggs form a continuous series, they can be separated with reasonable accuracy into three classes or types:

Type I.—Double-yolked eggs having the entire set of egg envelopes common to the two yolks.

Type II.—Double-yolked eggs having all or part of the thick albumen common to the two yolks, but with separate chalazal membranes.

Type III.—Double-yolked eggs in which the yolks have entirely separate thick albumen envelopes but a common egg membrane and shell.

RELATION OF THE NATURE OF THE DOUBLING OF THE EGG TO THE SEVERAL FUNCTIONAL DIVISIONS OF THE OVIDUCT

The most obvious interpretation of the various types of doubling observed in double-yolked eggs is that the two components may come together at any level of the oviduct from the ostium to the isthmus ring. The eggs classified as type I arise when the union of the two yolks occurs in that part of the duct where chalazal membrane is secreted (probably the funnel and funnel neck); type II, when the union is at any level in the albumen-secreting region more than the length of the first egg anterior to the isthmus ring; and type III, when the union occurs while the first egg is passing the isthmus ring.

Observations of the relation of the egg envelopes were made on nearly every double-yolked egg produced at the plant of the Maine Experiment Station during the past year. Data were collected on 131 eggs with two normal yolks and a common egg membrane and a shell. Only 21, or 16.03 per cent, were united in a common chalazal membrane, showing that they had passed practically the entire length of the oviduct together. In contrast to this, 93, or 70.99 per cent, had separate chalazal membranes but with all or part of their thick albumen common to the two yolks. These eggs showed every possible gradation from two yolks with entirely common thick albumen envelopes to cases where the two component eggs were contained in a very thin layer of common thick albumen. They had apparently come together at every possible level of the duct from a point in the funnel where the chalazal membranes are complete to a point near the lower end of the albumen-secreting region. Seventeen eggs, or 12.97 per cent, had entirely separate thick albumen envelopes. They had evidently come together while the first component was passing the isthmus ring.

The large proportion of double-yolked eggs with all or part of their thick albumen common to the two yolks is easily explained by the length of the portion of the duct in which they may come together. A photograph of the oviduct opened out to show the various regions is reproduced in Plate L. There is no visible line of demarcation between the funnel

(*A*), where the chalazal membrane and chalazas are probably secreted, and the albumen-secreting portion (*B*). There is, however, a difference in the general appearance of the glandular ridges and the microscopic character of the glands for a distance of 5 to 8 cm. in an adult laying barred plymouth rock fowl. There is a definite line of demarcation, the isthmus ring (*x*), where the egg membrane begins to be secreted. The length of the duct from the mouth of the funnel to the isthmus ring has been determined for 57 normal laying barred plymouth rock fowls. The mean length was 46.3 cm.

The portion of the oviduct in which two yolks can unite and have all the egg envelopes common is probably within the length of the funnel—that is, from 5 to 8 cm. The portion where they will have all their envelopes separate, except the egg membrane and shell, probably roughly approximates the length of the first egg when passing into the isthmus, or from 5 to 7 cm. The union of two eggs in any other part of the duct from the ostium to the isthmus ring (30 to 35 cm.) would result in the formation of a double-yolked egg with all or part of the thick albumen common to both yolks. If we take as the means of the above figures 7, 33, and 6 cm. and calculate the percentage that each is of the total length from ostium to isthmus ring we shall have the expected percentage of double-yolked eggs of each type, if the probability of the union of two yolks is equal at every level of the duct. The number and percentage of eggs of each type observed and the number and percentage expected on the above assumption are given in Table I.

TABLE I.—*Number and percentage observed of each type of double-yolked eggs and the number and percentage expected, if there is an equal probability of the union of the two components at every level of the oviduct*

Type.	Number.		Percentage.	
	Observed.	Expected.	Observed.	Expected.
I (all egg envelopes common)	21	19.94	16.03	15.22
II (separate chalazal membranes; all or part of thick albumen common)	93	93.98	70.99	71.74
III (separate thick albumen envelopes; common membrane and shell)	17	17.08	12.98	13.04
Total	131	131.00	100.00	100.00

The close agreement between the data for the eggs observed and those expected in each type supports the conclusion that the union of the component eggs occurs indiscriminately at all levels of the oviduct. While the eggs observed formed a graded series and the divisions of the duct are somewhat rough, nevertheless, variations in classification within any possible range of observation could not reverse the conclusion that the short distances of duct which could yield eggs of the first and

third type and the comparatively long portion where the union of two eggs could result in an egg of the second type are obviously in accord with the observed percentage of the eggs of each type.

THE QUESTION OF THE SIMULTANEOUS OVULATION OF THE TWO YOLKS OF A DOUBLE-YOLKED EGG

On purely theoretical grounds Parker [1] explains the origin of double-yolked eggs as the "simultaneous or almost simultaneous" discharge of two yolks from the same or separate follicles. He suggests that probably when the two yolks are inclosed in the same vitelline membrane they come from the same follicle, but that when they are in separate membranes they are probably from separate follicles.

Glaser [2] suggests that it is not necessary to assume simultaneous ovulation in the case of the two yolks of a double-yolked egg, since the first yolk may remain in the infundibulum until the next normal ovulation. The present author [3] has further suggested, first, that this delay of the first egg may occur at any level of the duct anterior to the isthmus ring; second, that the first egg may be moved back up the duct by antiperistalsis; and, third, that a yolk ovulated into the body cavity may be later picked up by the funnel and inclosed with its successor in a double-yolked egg.

It is probable that double-yolked eggs arise from some or all of these causes. However, as was pointed out in the previous paper,[3] at least an abnormally close succession of ovulations is necessary to account for a daily succession of double-yolked eggs or of double-yolked eggs laid after a series of normal daily eggs.

No bird belonging to the flock of the Maine Experiment Station during the last six years has produced double-yolked eggs on successive days, but a large number of fowls have produced double-yolked eggs within a series of normal daily eggs. For example, fowl No. 2K produced a double-yolked egg as the seventh egg of a 10-egg clutch, No. 37M one as the sixth egg of a 13-egg clutch, and No. 306K produced double-yolked eggs both as the second and fourth eggs of the same 6-egg clutch. In fact, in 43, or 36.44 per cent, of the 118 cases on which we have complete data the bird which produced a double-yolked egg had laid a normal egg on the preceding day. In these cases it seems certain that the period between ovulations must have been much shorter than the normal period. Further, in 17, or 14.40 per cent, of the 118 cases the bird laid normal eggs on both the preceding and following days. In these cases the evidence for a heightened rate of fecundity is unmistakable, although the ovulations which furnished the yolks for the double-yolked egg may not have been simultaneous.

[1] Parker, G. H. Double hens' eggs. *In* Amer. Nat., v. 40, no. 469, p. 13–25, 1 fig. 1906. Bibliography, p. 23–25.

[2] Glaser, Otto. The origin of double-yolked eggs. *In* Biol. Bul., v. 24, no. 3, p. 175–186. 1913.

[3] Curtis, M. R. Op. cit.

POSSIBILITY OF A RELATION BETWEEN THE RATE OF FECUNDITY AND THE TYPE OF DOUBLING OF THE DOUBLE-YOLKED EGG

It has been shown in previous paragraphs, first, that one-sixth of all the double-yolked eggs show by their structure that the two yolks have passed practically the entire length of the oviduct together (16 per cent have the complete set of egg envelopes common to the two yolks); and, second, that in more than one-third (36.44 per cent) of the cases of double-yolked eggs the two yolks must have been ovulated at an abnormally short interval, since these double-yolked eggs were laid on days following the production of a normal egg. Or, to put the matter in another way, in 43 out of 118 cases the two yolks must have been ovulated in less than the normal time, while in only 19 cases did the two yolks pass the entire length of the oviduct together. The analysis may be carried still farther: Of the 43 cases in which an egg had been laid on the preceding day only 7, or 16.28 per cent, were eggs with the two yolks inclosed in common chalazal membranes—that is, even where we have evidence from the egg record of the fowl that the ovulations must have been unusually rapid the structure of the egg indicates that they were simultaneous in only a small percentage of the cases. This is shown in the first column of Table II. This column also shows that in a few of these cases the entire thick albumen envelopes of the two yolks are separate. This indicates that the two eggs have not united until very near the end of the albumen-secreting portion of the oviduct.

TABLE II.—*Number and percentage of each type of double-yolked eggs occurring as single eggs, or preceded or followed within one day by a normal egg*

Type.	Cases in which a double-yolked egg occurred on the day following a normal egg.		Cases in which a normal egg was laid on the day following, but not on the day preceding the double-yolked egg.		Cases in which a double-yolked egg occurred as a single egg.		Double-yolked eggs observed.	Percentage of total.
	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Total number.	
I (all egg envelopes common)	7	36.84	6	31.58	6	31.58	19	16.10
II (all or part of thick albumen common)	33	39.76	20	24.10	30	36.14	83	70.34
III (all of thick albumen separate)	3	18.75	2	12.50	11	68.75	16	13.56
Total	43	36.44	28	23.73	47	39.83	118	100.00

It should be borne in mind that the structure of the egg can not be used to measure the time between ovulations. It only registers the level of the oviduct where the two eggs come together. Further, the author [1] has called attention to the fact that it is necessary to assume a difference in the rate of the passage of the two eggs through the oviduct, in order to account for the union of two yolks which did not enter simultaneously. Nevertheless, it seems certain that in cases where the eggs do not unite at the upper end of the duct the time of entrance of the two yolks must have been separated by a measurable period.

Table II also shows that some of each type of double-yolked eggs are produced as single eggs, some on the day after a normal egg was laid, and some on a day followed but not preceded by a normal egg. About one-third of the eggs of both type I and type II were produced in each of the three relations to the production of normal eggs, but more than two-thirds of the eggs of type III were single eggs.

This suggests that, while eggs of all types, including type III, may result from a heightened rate of fecundity, the most usual cause of the doubling of eggs at the end of the albumen portion is an abnormal delay of the first egg in the oviduct.

In this section it has been shown, first, that there is a certain, though small, percentage of the cases of double-yolked eggs in which, judging from the egg structure, the two yolks probably entered the oviduct practically simultaneously, and, second, that there is a still larger percentage of cases where, as shown by the egg records of the bird, the two ovulations must have occurred within a few (three or four) hours. It seems certain that in all cases of the simultaneous ovulation of two yolks the complete set of egg envelopes is common to the two. Yet neither the structure of the egg nor the egg record of the bird can prove absolutely that the time between ovulations has not been considerably reduced.

OVARIAN RELATION OF THE TWO FOLLICLES WHICH FURNISH THE YOLKS FOR A DOUBLE-YOLKED EGG

Glaser [2] described the pathological ovary of a bird which habitually laid double-yolked eggs. He concluded that in this case the double-yolked eggs arose from follicles secondarily fused. The secondary fusion of follicles resulted in a common blood supply which, when associated with a common state of permeability in the two ova, resulted in their simultaneous maturity and discharge. That the condition at autopsy of the ovary described by Glaser warrants the assumption that the secondary fusion of the follicles resulted in a common blood supply which was an important factor in the synchronous maturity and ovulation of yolks may, perhaps, be questioned. But at least it offers a suggestion in regard to the origin of double-yolked eggs which is open to investigation.

[1] Curtis, M. R. Op. cit.

[2] Glaser, Otto. Op. cit.

Some observations have been made at this laboratory upon the relation of the two follicles occurring in normal ovaries which have furnished the yolks for double-yolked eggs.

A young pullet, No. 1825 (1913 chick), laid her first egg at 1.30 p. m. on November 20, 1913. This egg was double-yolked. The bird was killed at 4 p. m. the same afternoon, and the ovary was carefully removed and photographed (Pl. LI, fig. 1). It was perfectly normal with a graduated series of seven enlarging yolks and two distinct follicles. There was no egg in the oviduct. The bases of the two follicles were separated by about 15 mm. of ovarian epithelium, which was covered with small yolks.

Another pullet, No. 8053 (hatched in 1913), laid a double-yolked egg at 3.30 p.m. on November 26, 1913. This was her first egg. At 4.35 p. m. the bird was killed. There was no egg in the oviduct. Two normal separate discharged follicles were present on the ovary (Pl. LI, fig. 2). The bases of the two follicles were quite distinct, although not situated at a great distance from each other. They were supplied by separate arteries from the same branch of the ovarian artery. The ovary was apparently normal in every respect. It contained a series of six enlarging yolks.

In both these cases the only yolks the bird had ever ovulated were the yolks contained in the double-yolked egg. In each case these yolks were from separate follicles which had separate blood supplies.

The study of the structure of the egg and of the egg record of the bird has already led to the conclusion that it is not necessary to assume simultaneous ovulation or even an unusually rapid succession of ovulations, except in a small percentage of the cases of double-yolked egg production. It, therefore, is important to consider these points in a study of the ovarian relation of the follicles.

In the two cases just discussed the double-yolked eggs were both of type II—that is, each yolk was inclosed in an envelope of thick albumen and then the two were inclosed in a very thin common envelope of thick albumen. Plate XLVIII, figure 1, is a reproduction of a photograph of one of these eggs, that of bird No. 1825. The doubling of these eggs had evidently occurred far down in the albumen-secreting region of the oviduct. We should not therefore expect that the two yolks had been simultaneously ovulated.

The cases in which there is good reason for suspecting simultaneous ovulations are those in which the two yolks are inclosed in a complete set of common envelopes. This shows at least that they have traversed the entire length of the oviduct together.

An egg of this type was produced on October 18, 1914, by bird No. 139M (Pl. XLVI, fig. 3). The common chalazal membrane has been partly torn away, in order to demonstrate that the two yolks have separate vitelline membranes. The bird laid a normal egg on October

19 and was killed and examined on the 20th. At the time of autopsy there was an egg in the oviduct. The egg record of this bird from October 1 to 20 is as follows:

October	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Eggs	1	...	1	1	1	1	...	1	1	1	(a)	...	...	1	1	1	...	b 1	1	c 1

a Nested, but did not lay. b Double-yolked egg. c Egg in oviduct at autopsy.

Plate LII, figure 1, shows the ovary of this bird. The ovary is perfectly normal, with a series of six enlarging yolks. As would be expected from the egg record, several discharged follicles were found, ranging in size, as in other normal ovaries, from the large one just discharged to those barely visible. The seven largest can be arranged according to size and associated with the eggs laid from October 14 to 20. Follicle A is much the largest and no doubt furnished the yolk found in the oviduct. Follicle B is next in size and evidently furnished the yolk for the egg laid on the 19th. Follicles C and C′ are practically equal in size and probably furnished the two yolks for the double-yolked egg on the 18th. Follicles D, E, and F continue the decreasing series and probably furnished, respectively, the yolks for the eggs on October 16, 15, and 14. All the other discharged follicles are distinctly smaller.

Every follicle on the ovary was carefully examined for evidence of the fusion of follicles or of a common blood supply. (The bird had been injected with starch solution.) All the follicles on the ovary had separate stalks and each had a single cavity. Follicles B, C, C′, D, and E, in common with all the other follicles in that part of the ovary, were supplied by separate small branches from a single large branch of the ovarian artery.

There is, then, in this case no evidence that the double-yolked egg has arisen from a fusion of follicles or from a common blood supply, although the structure of the egg indicates that the two yolks have passed the full length of the oviduct together.

Since there is evidence of simultaneous ovulations in less than one-sixth of the cases of double-yolked egg production and since even in such a case the two follicles may be quite distinct, two simultaneous ovulations resulting from the fusion of follicles are at least a very unusual cause for the production of a double-yolked egg.

NATURE OF THE FOLLICLE WHICH PRODUCED A LARGE YOLK WITH TWO GERM DISKS

A study of all the abnormal eggs produced at the Maine Experiment Station shows that the doubling of an egg in the ovary is rare. An egg belonging to this class was laid on March 1, 1914, by bird No. 311K. Externally this egg resembled a double-yolked egg. Its weight was 82.25 gm. It contained one single very large yolk (weight 30.12 gm.),

with two large and apparently normal germ disks (Pl. XLVI, fig. 1). There was a thin white line visible only part way around the yolk but passing between the two disks and standing out plainly in this region. The size of the yolk, the presence of two germ disks, and this line were the only evidences of doubling. The vitelline membrane was punctured and the yolk removed. The membrane was washed out carefully with salt solution. It contained a *single* cavity, with no suggestion of doubling, except the slight thickening seen as a white line on the surface of the yolk. The weight of albumen and shell, 44.47 and 7.66 gm., respectively, was comparable to the weight of these parts in a double-yolked egg where the two yolks are separate.

This egg clearly belongs to the first class described by Immerman [1] in which the two yolks are in a single vitelline membrane.

The bird which laid this egg was killed at 11 a. m. on the following day. A normal egg weighing 45.81 gm. was in the shell gland. The yolk of this egg was normal, having a single germ disk. It weighed 17.7 gm., which is about 59 per cent of the weight of the yolk with the two germ disks. The egg contained as yet only a small amount of thin albumen and no visible shell. It had evidently just entered the shell gland. The contained yolk had without doubt been ovulated the morning of the day the bird was killed, or about 48 hours after the ovulation of the yolk with the two germ disks. The egg would not have been laid until the second day following the one on which the large egg was laid.

The ovary of this bird is shown in Plate LII, figure 2. Two of the enlarging yolks have been removed, in order to show the follicles. Only three large follicles were present. Two of these, A and B, were about the same size, A being somewhat larger. The stalk of the third, which was considerably smaller, is seen at C. Either A or B must have furnished the yolk with the two germ disks. This yolk was nearly twice the size of the normal yolk in the oviduct. In the time which elapsed between the two ovulations the follicle which had produced this enormous yolk had been resorbed to practically the size of the one just ovulated. Neither of these follicles, nor in fact any other on the ovary, showed any evidence that it was composed of two fused follicles. If the double yolk arose from the fusion of two oöcytes, it seems probable that this took place at a stage earlier than the formation of the follicle.

The practically double size of the yolk contained in a single follicle but with two germ disks suggests that the germinal vesicle may be an important factor in determining the quantity of yolk deposited within a yolk membrane.

In the domestic fowl the fusion of follicles in an ovary capable of producing normal yolks must be of rare occurrence. The examination of several hundred ovaries of laying birds has not furnished a single case.

[1] Immerman, Ferdinand. Über Doppeleier beim Huhn. 43 p., 5 fig. Basel, 1899. Inaugural Dissertation.

In cases of general peritonitis accompanied by visceral adhesions, slight superficial adhesions are sometimes found between the follicles containing hardened yolk. These follicles could never produce normal yolks.

DESCRIPTION OF A FUSION OF YOLKS WHICH MAY HAVE ARISEN FROM A FUSED FOLLICLE

A small egg weighing only 19.88 gm. was laid on October 19, 1913, by bird No. 19K. This egg contained the double yolk shown in Plate XLVI, figure 2, and weighed only 1.45 gm. Neither part had a visible germ disk. The vitelline membranes were fused at the point of contact and there was a communication between the cavities of the two yolks, if they were actually separate yolks. The bird had been laying normal eggs and continued to do so. Why this very immature pair of yolks was ovulated is difficult to understand. Since the bird was not killed, the nature of the follicle which furnished them is not known.

This and the preceding case are the only ones which have come under our observation in which the two yolks were inclosed in a common vitelline membrane.

CONCLUSIONS

The various kinds of evidence given in this paper lead to the conclusion, first, that double-yolked eggs sometimes represent a heightened rate of fecundity and sometimes an abnormally low physiological tone of the oviduct; second, that, even in cases in which the rate of fecundity is high, the ovulations are not always simultaneous; third, from the above it is apparent that the production of a double-yolked egg can seldom be explained as a result of simultaneous ovulations; and, fourth, in cases in which we have the best of reasons for suspecting simultaneous ovulations, the two follicles may be quite distinct.

It seems quite possible that a heightened rate of fecundity may result in every conceivable shortening of the period between ovulations consistent with the daily rhythm in the general physiological activities of the bird. Whether it results in the formation of a double-yolked egg is no doubt determined by the actual length of the period and the following response of the oviduct.

SUMMARY

(1) Double-yolked eggs with normal separate yolks may have all the egg envelopes common to the two yolks, or they may have some separate and some common envelopes.

(2) They may be classified with reasonable accuracy into three groups:

Type I.—Double-yolked eggs having the entire set of egg envelopes common to the two yolks.

Type II.—Double-yolked eggs having separate chalaziferous layers but all or part of the thick albumen common to the two yolks.

Type III.—Double-yolked eggs in which the yolks have entirely separate thick albumen envelopes but a common egg membrane and shell.

(3) Of the eggs studied 16.03 per cent belonged to type I, 70.99 per cent to type II, and 12.98 per cent to type III.

(4) A large series of double-yolked eggs show all gradations within and between these groups.

(5) The most probable interpretation of this phenomenon is that the two components unite at any level of the oviduct from the funnel mouth to the isthmus ring.

(6) The conclusion that the union of the component eggs occurs *indiscriminately* at all levels of the oviduct is strongly supported by the fact that the percentage of eggs of each type is closely porportional to the percentage of the portion of the duct in which the union of two eggs would give double-yolked eggs of that type.

(7) In 36.44 per cent of the double-yolked eggs the ovulations which furnished the two yolks must have been separated by an abnormally short interval, since a normal egg had been laid on the preceding day.

(8) An examination of the egg structure, however, shows that the two yolks have passed the entire length of the duct together in only 16.28 per cent of the cases in which the ovulations are known to have been usually rapid.

(9) While a heightened rate of fecundity may result in the production of an egg of any of the three types, 68.75 per cent of the eggs of type III are single eggs. It seems probable that many of them have resulted from the delay of the first egg in the oviduct.

(10) The ovary of each pullet which had just laid a double-yolked egg as her first egg contained two normal separate follicles which had separate blood supplies. In these cases, however, the doubling of the egg had occurred near the end of the albumen-secreting region.

(11) In a case in which there was evidence from the structure of the egg that the two yolks had passed the entire length of the oviduct together the two follicles were also quite distinct, with separate blood supplies.

(12) This, together with the fact that in only a small percentage of double-yolked eggs is there any evidence of simultaneous ovulation, indicates that the fusion of follicles and a resulting common blood supply is by no means the usual cause for the production of a double-yolked egg.

(13) A simple normal follicle furnished the yolk with two germ disks; hence, the fusion of the oöcytes (if this was the origin of the two germ disks) must have occurred before the formation of the follicle.

PLATE XLVI

Fig. 1.—Large yolk (weight, 30.12 gm.) with two germ disks; found in a large hen's egg.

Fig. 2.—Fused immature yolks (weight, 1.45 gm.); found in a small hen's egg.

Fig. 3.—Type I double-yolked egg, showing two yolks with separate vitelline membranes but inclosed in a common chalaziferous layer. The yolks were slightly pressed apart before photographing, in order to show that the vitelline membranes were entirely separate.

1

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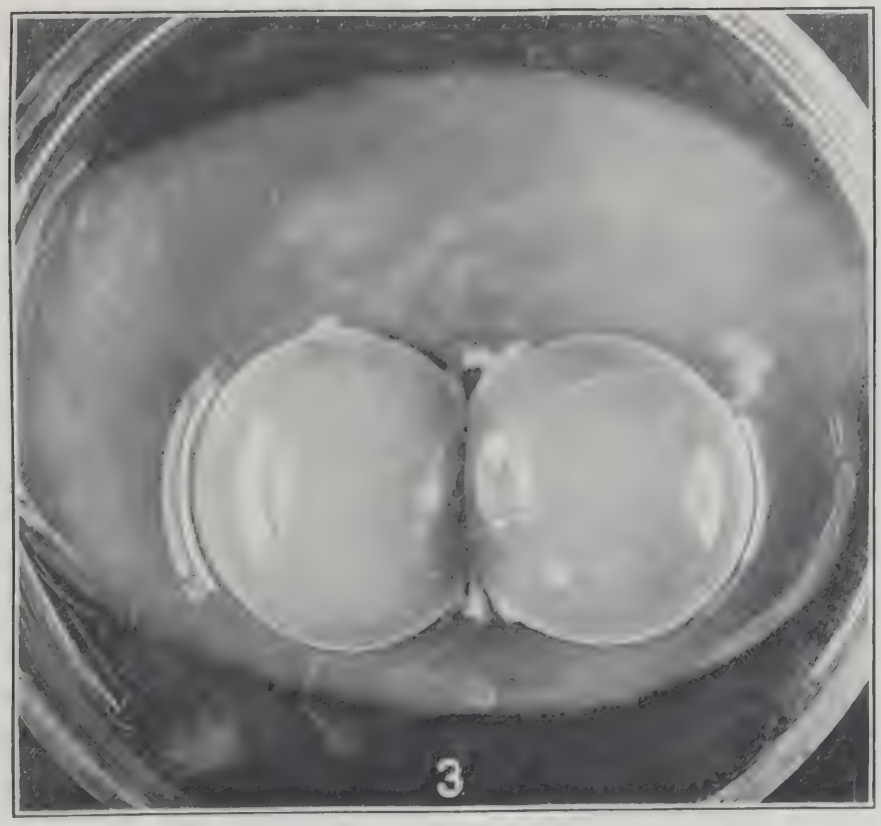

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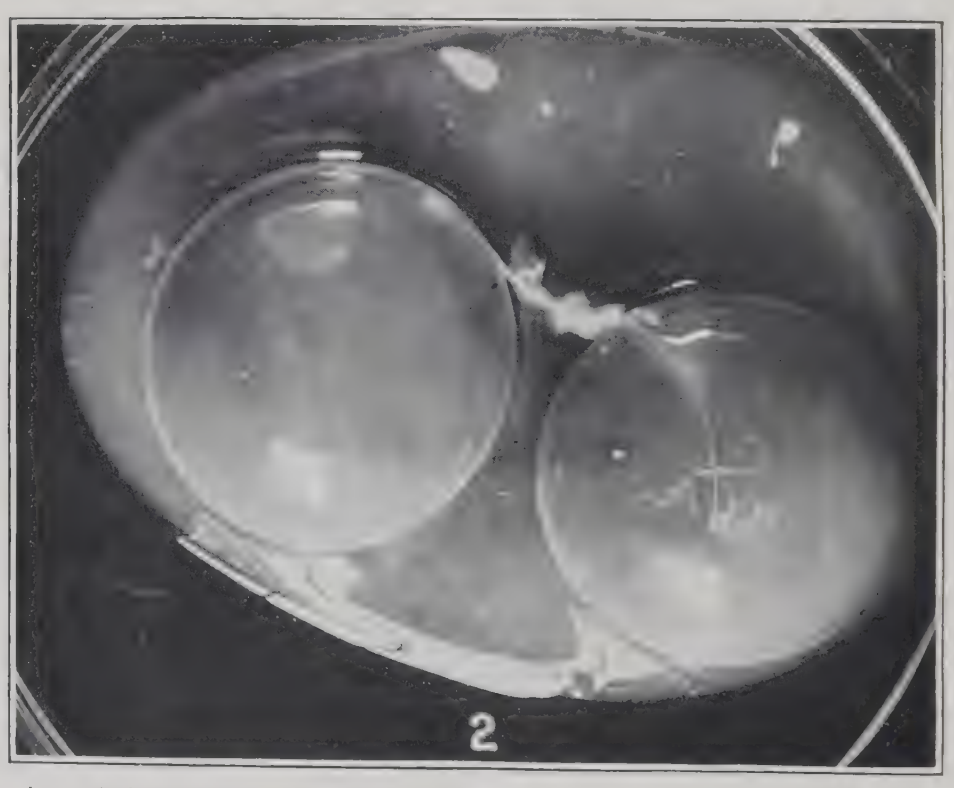
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PLATE XLVII

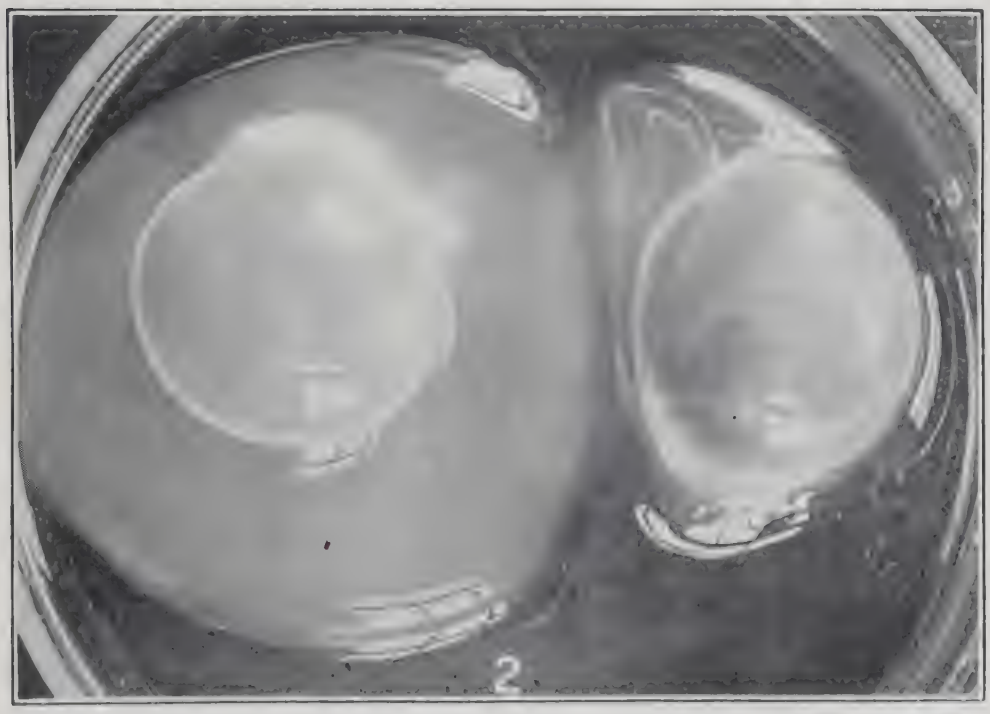
Fig. 1.—Type I double-yolked egg, showing two yolks with separate vitelline membranes but inclosed in a common chalaziferous layer. The yolks were slightly pressed apart before photographing, in order to show that the vitelline membranes were entirely separate.

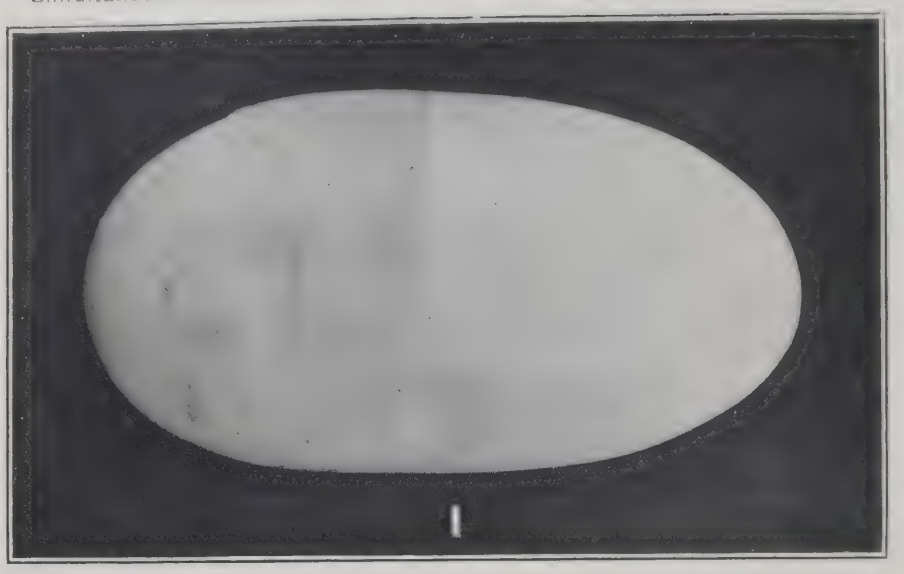
Fig. 2.—Type II double-yolked egg, showing two yolks with separate chalazal membranes but common thick albumen.

PLATE XLVIII

Fig. 1.—Type II double-yolked egg, showing two yolks with some separate and some common thick albumen envelopes.

Fig. 2.—Type III double-yolked egg, showing two yolks with all the thick albumen separate.

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2

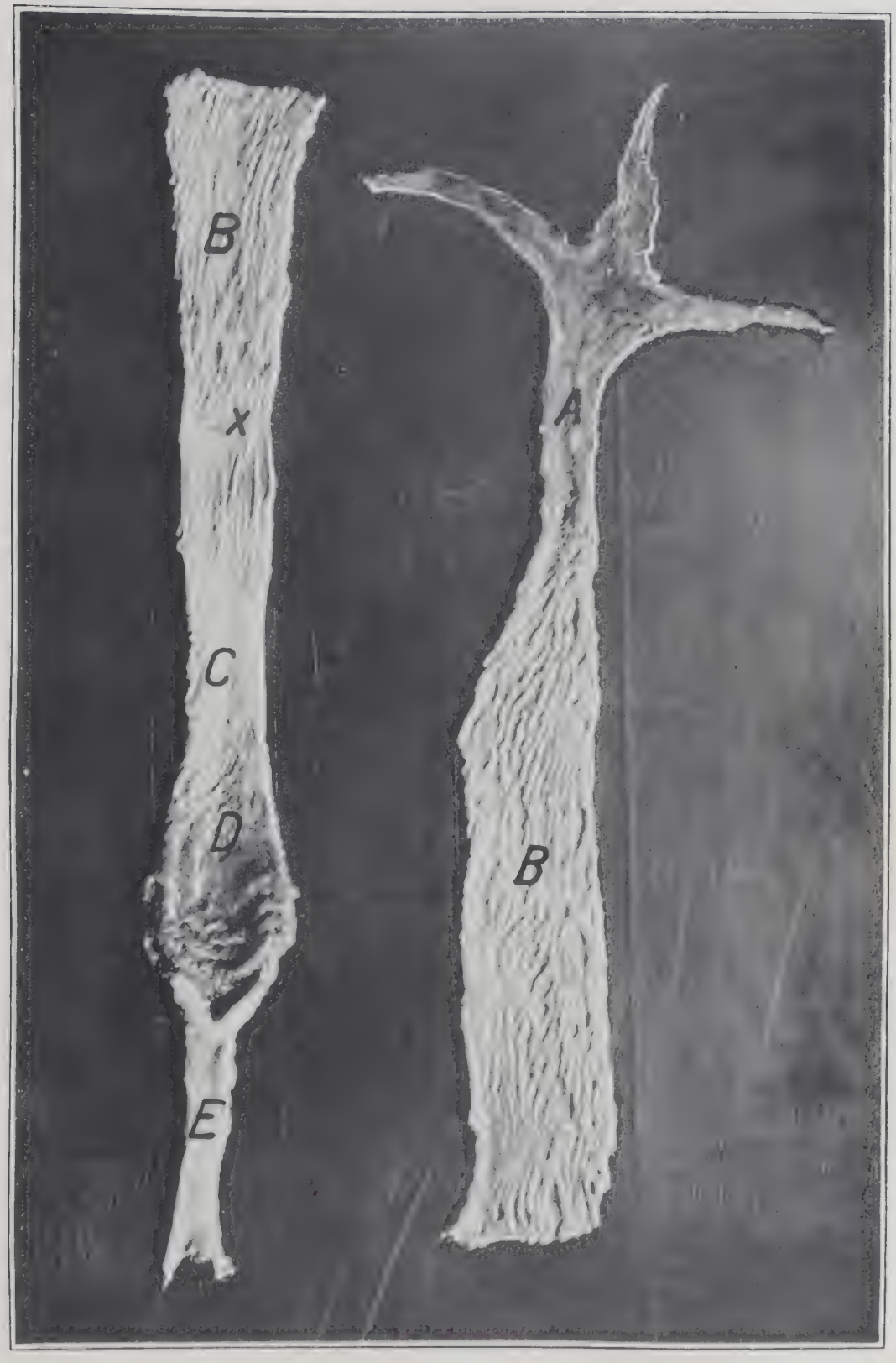
PLATE XLIX

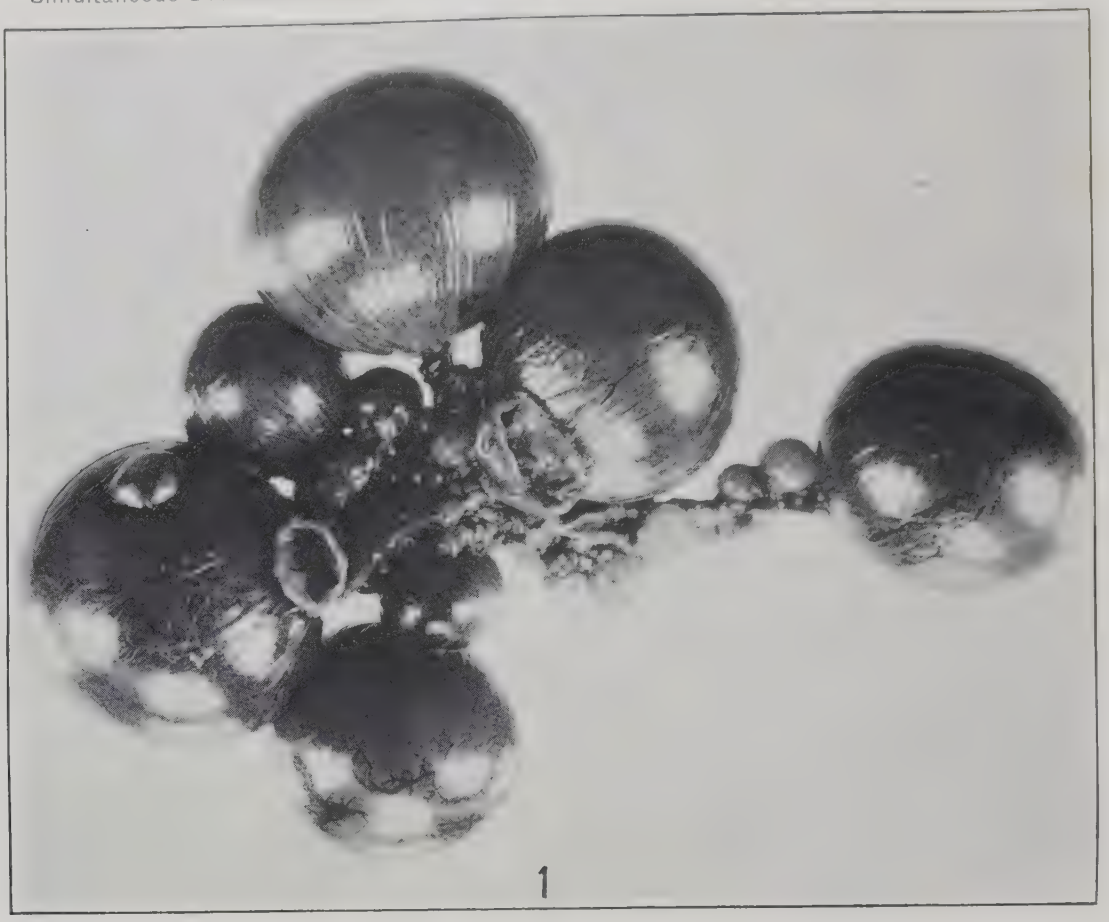
Fig. 1.—Shell of type III double-yolked egg, which shows external evidence of its double nature by a seam in the shell.

Fig. 2.—The inside of the shell shown in figure 1, showing the fold of egg membrane which projected between the two component eggs.

PLATE L

Oviduct removed from a laying bird and cut open along the point of attachment of the ventral ligament. It is opened back, showing the characteristic glandular regions. *A*, Funnel; *B*, albumen-secreting region; *X*, isthmus ring; *C*, isthmus; *D*, shell gland; and *E*, vagina.


B
x
C
D
E
A
B



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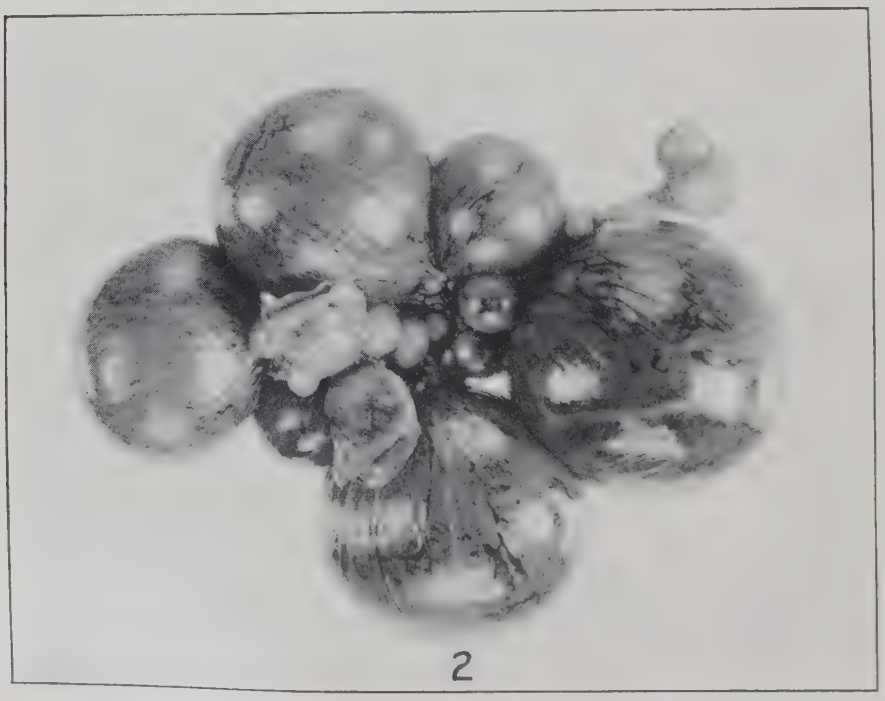

2

PLATE LI

Fig. 1.—Ovary of a pullet, showing the follicles which produced the yolks for the double-yolked egg shown in Plate XLVIII, figure 1.

Fig. 2.—Ovary of a pullet, showing follicles which produced the yolks for a double-yolked egg similar in structure to the one shown in Plate XLVIII, figure 2.

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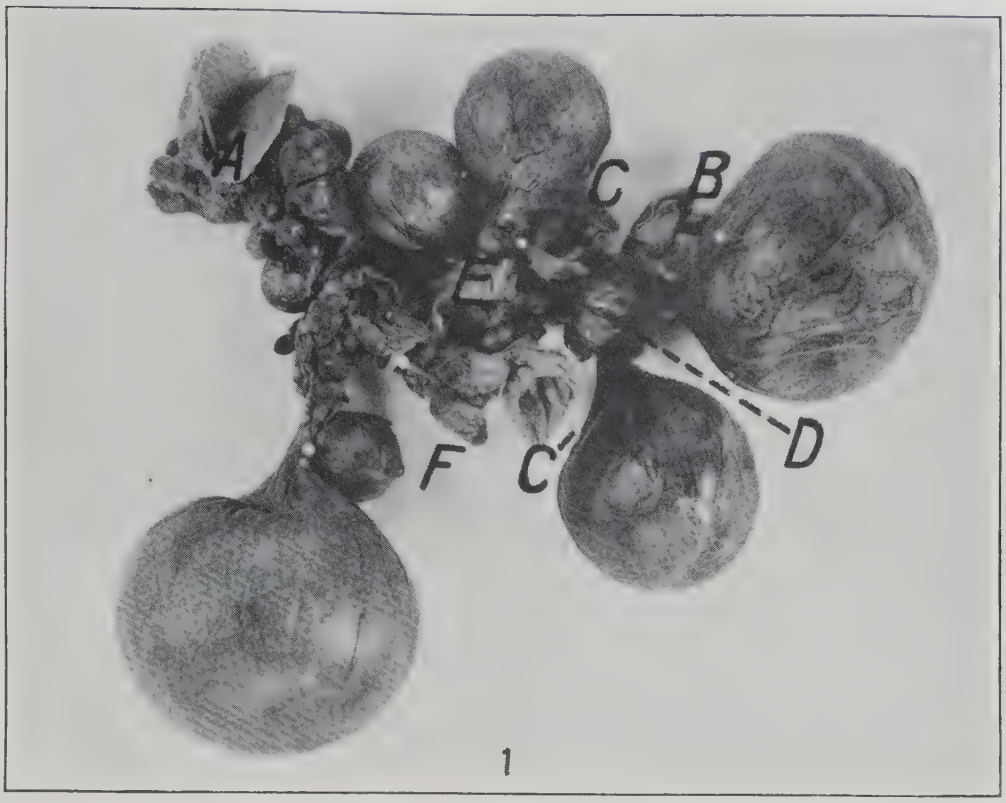
PLATE LII

Fig. 1.—Ovary of a pullet, showing a series of resorbing follicles, two of which (probably *C* and *C'*) produced the yolks for the double-yolked egg shown in Plate XLVI, figure 3.

Fig. 2.—Ovary of a bird, showing the two largest resorbing follicles, one of which produced the yolk with two germ disks shown in Plate XLVI, figure 1.



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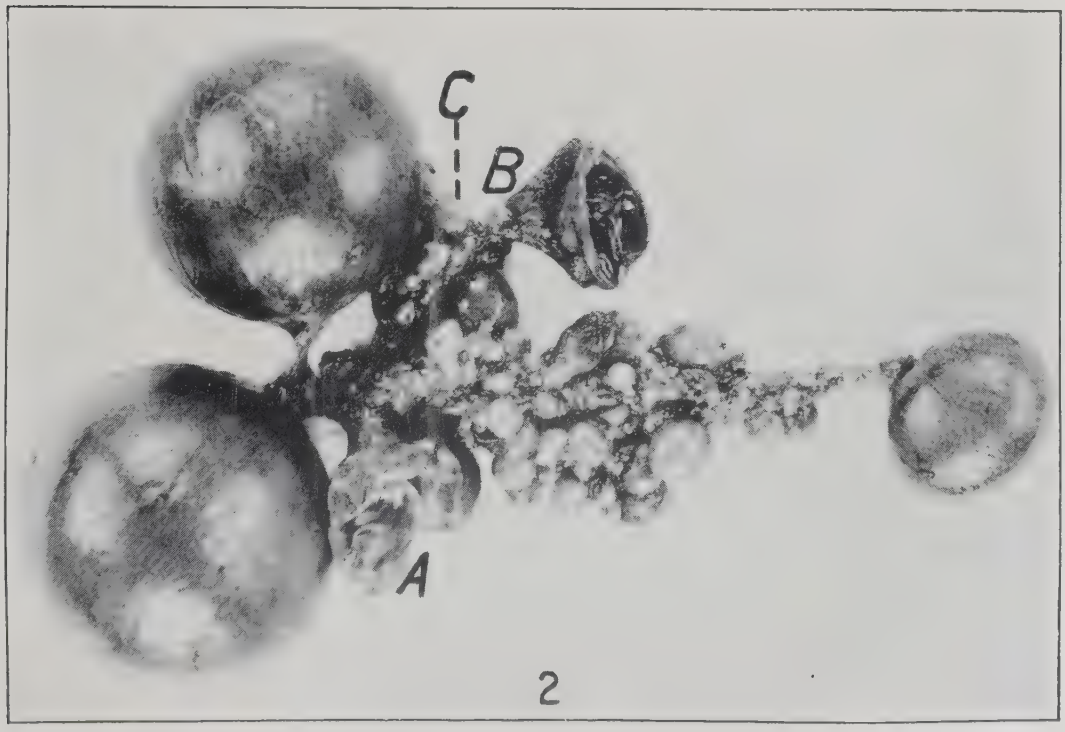

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BRACHYSM, A HEREDITARY DEFORMITY OF COTTON AND OTHER PLANTS

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The word "brachysm" is suggested as a name for abnormal variations of plants characterized by shortening of the internodes, without corresponding reductions of other parts. Brachysm is to be distinguished from nanism, or true dwarfing, which involves proportional diminutions of many parts, if not of all. Genuine dwarfs, with consistent reductions of all of the organs of the plant, would have little value for agricultural purposes, but many brachytic varieties are highly prized. In spite of their shorter internodes, brachytic varieties often bear leaves, flowers, and fruits as large as plants of normal stature, and sometimes larger. Most of the so-called "dwarf," or "bush," varieties of peas, beans, squashes, tomatoes, and other garden vegetables represent brachysm rather than true dwarfing. The "cluster" and "limbless" varieties of cotton belong to the same category, and the San Ramon coffee of Costa Rica affords another example of brachysm in a woody plant.

A special interest may be claimed for the cluster and limbless varieties of cotton because they seem to throw light on the nature of brachysm and similar abnormalities as phenomena of heredity. As brachytic varieties arise by mutation and show alternative inheritance in crosses, they illustrate two of the phenomena of heredity that have received much attention in recent years. The morphological and physiological relations of such characters must be understood before it is possible to appreciate their practical importance in breeding or their bearing upon general evolutionary problems.

SPECIAL FEATURES OF BRACHYSM IN COTTON

The special interest that attaches to the phenomena of brachysm in cotton arises from two general facts: That the shortening of the internodes is usually confined to the fruiting branches and that it is usually accompanied by other abnormalities. On account of the specialized structure of the cotton plant, it becomes possible to learn more of the relations of brachysm to other phenomena of heredity than if all of the internodes of the plant were affected and the other organs remained unchanged.

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The cotton plant has a definite dimorphism of branches, no flowers or bolls being produced on the main stalk or the vegetative branches.[1] The main stalk and the vegetative branches of cluster cottons are not shortened, but are often longer in brachytic varieties than in those that have normal fruiting branches. The leaves of the main stalk and vegetative branches of cluster cottons are of normal form, but they are larger, of thicker texture, and have longer petioles than those of normal long-jointed varieties. The axillary buds of noncluster varieties usually remain dormant or produce long vegetative branches, but in cluster cottons they commonly develop into short branches and produce one or two bolls. These differences may be considered as direct results of the failure of the fruiting branches to make normal growth, for similar changes occur in noncluster varieties, in plants that have been severely pruned, and also in seedlings that lose their terminal buds through insect injuries or by abortion.[2]

The abnormalities that accompany the shortening of the fruiting branches in cotton are shown in the forms of the leaves and the involucral bracts. The leaves of short-jointed fruiting branches often become smaller and more bractlike, while the bracts are often enlarged and leaflike, and show definite indications of the stipular and foliar elements that are completely fused in normal bracts.

Each of the involucral bracts of a cotton plant represents a modified leaf. The specialized form of the bract results from having the stipules of the leaf greatly enlarged, the petiole entirely suppressed, and the blade reduced and united with the enlarged stipules. The abnormal leaflike bracts and bractlike leaves of cluster cottons show all the stages between normal leaves and normal bracts. In such abnormalities there is usually an obvious relation between the reduction of the blade or the suppression of the lobes of the blade and the enlargement of the stipules of the same leaf, and also between abnormalities of the leaf and of the involucral bracts of the same internode. This can be understood by comparing Plate LIII, which shows an abnormal reduced leaf and an abnormal enlarged bract, with Plate LIV, which shows a leaf and a bract of normal size and proportions. The converse relation is that when the bracts take a more leaf-like form, with an enlargement of the middle or blade element of the bract, it is almost always accompanied by a reduction of the stipular elements, as shown in the leaf-like bract in Plate LIII. When one side of a leaf is reduced or has the lobe sup-

[1] The dimorphic specializations of the branches and leaves of the cotton plant have already been described. (Cook, O. F. Dimorphic branches in tropical crop plants ... U. S. Dept. Agr. Bur. Plant Indus. Bul. 198, 64 p., 9 fig., 7 pl. 1911. Cook, O. F. Dimorphic leaves of cotton and allied plants in relation to heredity. U. S. Dept. Agr. Bur. Plant Indus. Bul. 221, 59 p., 18 fig., 5 pl. 1911.)

[2] Abortion of terminal buds is a frequent result of a peculiar disorder of cotton seedlings previously described. (Cook, O. F. Leaf-cut, or tomosis, a disorder of cotton seedlings. *In* U. S. Dept. Agr. Bur. Plant Indus. Circ. 120, p. 29–34, 1 fig. 1913.)

pressed, as in Plate LV, the stipule on the same side of the leaf shows a corresponding enlargement.[1]

That brachysm in cotton is confined to the fruiting branches is doubtless connected with the fact that the fruiting branches have more direct relations with the floral organs. On account of the definite dimorphism of the branches of the cotton plant, no floral buds are produced on the main stalks or the vegetative branches. A study of brachysm in cotton seems to indicate that such variations represent intermediate stages or combinations of floral and vegetative characters. Brachytic varieties have leaves that are more like involucral bracts, and bracts that are more like leaves than those of normal long-jointed varieties. From this point of view it is easy to understand that leaves of the fruiting branches would be more likely to show abnormal anticipations of the characters of the floral bracts than the leaves of the vegetative branches or the main stalks, for these have no floral buds and represent earlier stages in the development of the plant.

INDEPENDENT ORIGINS OF BRACHYTIC VARIATIONS

It has been supposed that all of the "cluster" varieties of cotton belong to the same botanical series, but in reality the possession of short-jointed fruiting branches is not a reason for supposing that two varieties are related. Brachytic variations are very frequent and have been found in so many different species and varieties of cotton that the idea of derivation by crossing with a brachytic ancestral type is unwarranted.

Brachysm is not known as a normal character of any wild species of cotton, but seems to follow as one of the results of domestication and selection. The same is apparently true in other families of plants. Brachytic variations have arisen independently in several different types of peas, beans, squashes, melons, and other climbing and creeping plants. It is in such plants that the abnormal nature of brachytic variations is most obvious. Under natural conditions, short-jointed variations of climbing plants would be placed at a still greater disadvantage than those of shrubby plants like cotton or coffee.

If brachytic variations be supposed to represent the formation of a new character, as assumed in the mutation theory of De Vries, it is difficult to understand why the same character should arise suddenly in so many different and unrelated types of plants. But if brachysm be considered as a defect or failure of normal heredity, it becomes easier to understand that many kinds of plants might be subject to similar derangements. That the brachytic tendencies should appear independently in so many different genera and families of plants makes it reasonable to look for a general interpretation of this class of abnormal variations.

[1] A further account of these abnormalities, with figures of some of the intermediate forms of bracts and leaves, may be found in an earlier bulletin. (Cook, O. F. Heredity and cotton breeding. U. S. Dept. Agr. Bur. Plant Indus. Bul. 256, p. 69–78, fig. 3–12, pl. 4–6. 1913.)

DIFFERENT DEGREES OF BRACHYSM

The interest of brachytic variations as a phenomenon of heredity is increased by the fact that the tendency is manifested in many different degrees. The most extreme form of brachysm is represented by a complete abortion of the fruiting branches, but there is an apparently complete series of stages from these completely sterile monstrosities to the normal long-jointed forms. The strictly cluster or limbless forms have the fruiting branches reduced to a single joint or a few very short joints, as shown in Plate LVI. From this extreme condition the intermediate stages run through the ordinary cluster and semicluster types to those that show no reduction of the joints of the fruiting branches. Even in the same field of cotton it is often possible to find a wide range of variations in the lengths of the internodes of the fruiting branches, especially in varieties like the King, that produce many brachytic variations.

In some varieties, such as the Triumph cotton of Texas, the lower fruiting branches often show a tendency to brachysm not shared by branches farther up. Other varieties show the opposite tendency to form long-jointed fruiting branches on the lower part of the stalk and short-jointed branches on the upper part. Some varieties that usually have long-jointed fruiting branches show the cluster tendency when the growth of the plants is restricted by unfavorable conditions, while other varieties are always short-jointed. There may also be pronounced irregularities in the lengths of the joints, even on the same fruiting branch. (See Pls. LVII and LVIII.)

In addition to the fundamental difference between the vegetative and fruiting branches, the basal internode of the fruiting branches is generally longer than the others and often maintains its length when the others are shortened. In a peculiar variety of Egyptian cotton grown in experimental plantings at Bard, Cal., under the name of Dale, most of the fruiting branches develop only this long basal internode, and produce only a single boll, the other internodes being aborted. (See Pl. LXII.) When the leaves, buds, and joints of such branches are suppressed, the boll appears to be borne on a greatly elongated pedicel.

The extent to which these abnormalities are often carried may be more easily understood by reference to the photographs of the plant shown in Plate LXI, figure 1. The main stalk was rendered completely sterile by the abortion of all of the fruiting branches, though the vegetative branches of the same plant produced a few fruiting branches and ripened a few bolls. Finally the growth of the main stalk was stopped by the abortion of the terminal bud. (See Pl. LXI, fig. 2.) In more normal plants of this variety the single-jointed fruiting branches are often accompanied by one or two other branches of similar form arising from the axillary bud. (See Pl. LXII.) In an extreme case like that shown in Plate LXI, the axillary buds abort, as well as the extra-axillary buds

that normally produce the fruiting branches. The regular abortion of these axillary buds renders it the more probable that the abortion of the terminal bud was not accidental. The case shown on the left-hand figure of Plate LXII, the transformation of the terminal bud into a flower bud subtended by an abnormal leaf, affords still more definite evidence of abnormality.

SHORTENING OF INTERNODES BY DROUGHT

The internodes are always longer under conditions that favor luxuriant growth of the plants than where growth is restricted by drought. Though this relation is general, some varieties shorten their internodes much more than others. The susceptibility to shortening is sometimes so great that the same variety may be short-jointed like a cluster cotton in some places, while under other conditions it behaves as a normal long-jointed variety. Attention was called some years ago to a case of this kind in a variety called the Parker that had been grown in Texas for several seasons as a long-jointed variety, but behaved as a short-jointed or semi-cluster variety at Del Rio in the season of 1907.[1]

The difference between the true brachysm and this false brachysm induced by changes of external conditions is that the latter is not inherited. The false brachysm represents an adaptive change or accommodation to the conditions instead of a definite alteration of the expression relations of the characters. When whole stocks of plants or animals respond in the same way to a change of external conditions, the changes are usually in the nature of accommodations and are readily reversible. But the possibility that an increase in the number of heritable variations toward brachysm might be induced by environmental shortening of the fruiting branches would be worthy of investigation. This possibility is suggested by the fact that individual variations in the direction of the cluster habit appear more frequently in some localities than in others in the same variety of cotton. Thus, it was noticed in the season of 1913 that plants of the cluster form were of frequent occurrence in many fields of Durango cotton in the Imperial Valley of California, whereas in a field of Durango cotton at Deep Creek, Va., no cluster plants could be found.

Other illustrations of the influence of external conditions are afforded by irregularities in the lengths of the internodes of the same plant, or even of the same branch. (See Pl. LVII and LVIII.) In such cases the

[1] "The Parker variety of cotton showed a pronounced semicluster habit or shortening of the internodes of the fruit branches, a notable departure from the previous behavior of our stock of this variety, which had been under observation in several different localities in the preceding years. Apart from the fact that every precaution is taken to avoid mistakes in labeling and planting the seed, the possibility of error in this case seems to be entirely eliminated by the fact that six different selections of Parker were grown at Del Rio and that all of them behaved in the same manner with reference to this change in the lengths of the fruiting branches. Plantings of the same strains from the same lots of seeds in several other localities in Texas in the same season produced no such results." Cook, O. F. Suppressed and intensified characters in cotton hybrids. U. S. Dept. Agr. Bur. Plant Indus. Bul. 147, p. 20. 1909.

environmental shortening or false brachysm is often accompanied by other abnormalities, like the inherited form of brachysm. On a short internode intercalated between two of normal length the pedicel is likely to have a sloping or decurrent base, with the end of the internode prolonged beyond the insertion of the pedicel, though usually not so much as in cluster cottons.

This irregularity is less difficult to understand when it is remembered that the floral bud of each internode develops in advance of the growth of the next internode. On account of this sequence of development, two structural elements may be recognized in the internodes, the tissues that are developed early to support the pedicel and those that develop somewhat later in connection with the next internode. If a change to more favorable external conditions occurred during the growth of an internode, its effect must be greater upon the part of the internode that is the last to reach its full development. Thus, in connection with the development of a longer internode next to a short one, a slight elongation of the supporting part of the short internode would be induced, and a resulting tension between the two sides of the internode. This would account for the tearing of the tissues at the base of the pedicel, which often occurs. Hence, we see that a part of the abnormality of the internodes that accompanies brachysm may be capable of a simple mechanical explanation, as arising from changes in external conditions during the period of development of the affected internodes.

RETENTION OF BLASTED BUDS IN BRACHYTIC VARIETIES

The facts of brachysm in cotton seem to indicate that the shortening of the internodes of the fruiting branches is in the nature of a premature or accelerated expression of the floral character. This view seems preferable to the idea that a new character has been substituted for an old one in the mechanism of transmission, as usually assumed in theories of mutation and Mendelism. That the shortened internodes partake of the nature of the pedicels of the flowers is indicated not only by their reduced length but by the fact that they are often completely fused with the pedicels. At the base of a normal pedicel is a joint or layer of specialized tissue indicated externally by the absence of hairs and oil glands from the surface, but in cluster cottons the formation of this specialized layer is irregular. It is one of the recognized peculiarities of cluster cottons that abortive buds and those that are infested by boll-weevil larvæ often remain attached to the plant, whereas in varieties with normal fruiting branches the blasted buds are soon shed. The lack of definite differentiation between the pedicels and the joints of the branches is responsible for the more frequent retention of the buds in cluster varieties.

Normal shedding of the buds takes place by the formation of a circular fissure at the base of the pedicel, the subsequent wilting of the bud, and

the breaking away of the central pith of the joint. The circular fissure is formed just above the slight ridge or rim that connects the base of the stipules, the position being marked in advance, as already stated, by a narrow zone of smooth skin without any of the hairs and oil glands that are scattered over all of the neighboring surfaces.

The less definite differentiation of internodes and pedicels in cluster varieties often interferes with the formation of a normal circular fissure for the shedding of the abortive buds, which then remain hanging on the plant. The lower side of the base of the pedicel, the side that faces the main stalk of the plant, is found to be more or less confluent with the surface of the supporting internode. The zone of smooth tissue that indicates the position of the fissure, instead of being circular, may extend far down on the internode; or all indication of a fissure zone may be lacking, so that the pedicel appears as a direct continuation of the internode. The result of such malformations is that the blasted buds, instead of promptly falling off, turn brown and shrivel while still attached to the plant.

The casual observer is likely to suppose that the shriveled buds have been stricken by a blight, and there is often a strip of dead tissue running down on the internode from the base of the withered bud as though a bacterial or fungous disease were extending along the branch. Though often mistaken for a diseased condition, such injuries are merely the mechanical consequences of the abnormal structure of the internode and the failure to form a properly specialized joint between the internode and the pedicel of the floral bud. The formation of the joint is usually indicated, for the death of the epidermis commonly follows a definite line, even when the bud does not drop off. A complete separation of the underlying tissues allows the shriveled bud to fall away eventually, leaving a long scar extending down the internode, instead of a normal circular scar at the end of the internode. (See Pl. LVIII.)

Decurrent pedicels are not confined to brachytic varieties, but are often found in abnormal individuals of long-jointed varieties, though usually the internodes that have the decurrent pedicels are shorter, and other indications of abnormality may be present. Thus, in connection with the examples of decurrent pedicels shown in Plate LVIII there is an abnormal inequality in the lengths of the internodes, one being about five times as long as the others.[1]

MORPHOLOGY OF DECURRENT PEDICELS

As already noted, extreme cases are sometimes found in which the pedicels are not only decurrent upon the internodes but seem to lose their

[1] A somewhat different interpretation of the decurrent pedicels of the cotton plant is presented by Prof. Francis E. Lloyd. (Lloyd, F. E. Abscission. *In* Ottawa Nat., v. 28, no. 3/4, p. 41–52, 3 fig.; no. 5/6, p. 61–75. 1914.)

terminal positions and to arise from intermediate points. Examination of such cases at first suggested the idea that the floral bud might belong morphologically to the next internode below. In this view the pedicel would be merely coalesced with the supporting internode, instead of being produced from it. The branch morphology of the cotton plant is unusually complicated on account of the dimorphism of the branches and the extra-axillary position of the floral buds and of the buds that give rise to the fruiting branches.[1]

The assignment of the floral bud to the internode below seems to be forbidden by the fact that when the base of the pedicel becomes decurrent upon the supporting internode the stipule that subtends the pedicel and the stipular rim that incloses the base of the pedicel also become elongated and decurrent along the side of the internode. Thus, instead of merely a lower insertion of the flower bud, the whole internode is modified, and the nature of the modification makes it clear that the floral bud is borne normally above the stipular rim.

Though other families of plants afford instances where flower stalks or floral branches remain united with the basal portion of the next internode, it is very difficult to believe that an adnate or coalesced pedicel would be able to surmount the stipular rim and climb, so to speak, into the axil of the leaf of the internode with which it had become coalesced. In rare cases the pedicel of a cotton boll is joined to the internode above, but the result is clearly abnormal, and lends no support to the theory of coalescence of pedicels and internodes as a normal condition. (See Pl. LX.) A pedicel that is united with an internode is usually longer than the normal pedicels, while the internode is shorter than the others and is turned from its normal position to follow the direction of the pedicel to which it is attached. There is no tendency in these adherent pedicels to form an angle or a joint at the end of the internode, as might be expected if the theory of coalescence were correct.

The presence of a flower bud on the basal internode of fruiting branches offers another difficulty under the theory of coalescence. If it were assumed that each floral bud belongs, not to the supporting internode, but to the one lower down, it would necessarily follow that the floral bud of the basal joint of a fruiting branch could not belong morphologically to the fruiting branch at all, but must be assigned to the main stalk or the vegetative branch from which the fruiting branch is produced. This supposition would add new elements of complexity to the structural morphology of the cotton plant, already sufficiently complicated.

BRACHYSM ACCOMPANIED BY FASCIATION AND ADHESION

Further reasons for looking upon brachysm as a failure of normal differentiation of parts are found in the other abnormalities of the

[1] Cook, O. F. Morphology of cotton branches. *In* U. S. Dept. Agr. Bur. Plant Indus. Circ. 109, p. 11–16. 1913.

branches that often accompany brachytic variations and are of very frequent occurrence in cluster varieties. Fasciation, or duplication of parts, appears in many different stages, ranging from simple forking of internodes or pedicels to inclusion of two flowers in the same involucre, or two bolls in the same calyx, or to an abnormal increase of the number of carpels.[1]

Sometimes the fasciated internodes remain united for their whole length and bear two leaves at the end, like the internodes of opposite-leaved plants. In rare cases double internodes bear two flowers or bolls, though usually the floral buds of abdominal internodes are aborted. (See Pl. LIX.)

Union between the pedicel of a boll and the next internode of the fruiting branch is another abnormality to which reference has already been made. Adhesion occurs in connection with brachysm, though it also appears occasionally in connection with short joints in noncluster varieties. It is more likely to be noticed in such cases because of the contrast with the normal joints of the branch. Doubtless as a result of the fact that the pedicel of the floral bud develops normally in advance of the next joint of the branch, these adherent internodes are bent upward in the direction of the pedicels to which they are joined and form a distinct angle or elbow with the preceding internode of the branch. (See Pl. LX.) It is evident in such cases that the abnormality involves something more than a mere adhesion of the epidermal tissues, for the affected internodes are much shorter than the others, and even shorter than the pedicel to which they are united. In most cases the short joints lose their floral buds or young bolls by abortion.

ANALOGY BETWEEN BRACHYTIC VARIATIONS AND HYBRIDS

The fact that the shortening of the internodes is so often accompanied by abnormal leaves and involucres suggested the view here advanced, that brachysm represents a failure to maintain the normal specializations of the parts. Considered as examples of intermediate expression of characters, the shortened internodes and abnormal involucres of the cluster cottons afford a suggestive analogy with the abnormal intermediate forms of branches and involucres that often appear in hybrids between diverse species of cotton. This analogy may be supported by the fact that sterility, or blasting of the buds or the young bolls, is very frequent in brachytic varieties of cotton, as well as in hybrids, and is especially common in involucres that have the abnormal intermediate forms of bracts.

The idea that short-jointed variations differ from the parent stocks in only this one character, as often assumed by writers on Mendelism, is

[1] Reasons for looking upon fasciation as a symptom of degeneration have been given by Thomas Meehan. (Science, v. 3, no. 70, p. 694. 1884.) Meehan concluded that "a fasciated branch is an imperfect and precocious attempt to enter on the flowering or reproductive stage."

evidently not true of brachytic variations of cotton. While it may be that the mutations of other plants do not show changes in so many characters in connection with brachysm, the general fact seems to be that mutative changes affect several characters at once, instead of one character alone. It is possible, of course, to disregard the other differences and thus simplify the discussion of the Mendelian phenomena by giving exclusive attention to single differences, but many of the statements that have been made give a very misleading idea of the nature of mutative variations.[1]

In normal cotton plants there are sharp contrasts in length between the internodes that form the joints of the fruiting branches and those that form the pedicels of the flowers, just as there are definite differences in form and structure between the foliage leaves and the specialized leaves that constitute the involucre. In brachytic varieties these contrasts become less marked. The changes of characters are all in the direction of reduced specialization of internodes. Instead of the normally contrasted expression of the characters of the different kinds of internodes represented in the normal branches and floral parts, there is a reduced contrast or intermediate expression of the characters, resulting in the formation of abnormal internodes. Variations of this kind, resulting from intermediate expressions of characters that are normally distinct or separate in expression, may be described as metaphanic variations. These would form a general class of abnormalities to include brachysm and other similar aberrations of heredity.

BRACHYSM AND HOMOEOSIS

The nearest approach to a recognition of metaphanic variations as representing intermediate expression of characters, as a general factor in heredity, is probably to be found in the theory of translocation of characters, or homoeosis, brought forward a few years ago by Dr. R. G. Leavitt. The theory of homoeosis is that "a character or a system of organization which has been evolved in one part of the body is transferred, ready-made, tc another part." [2]

The theory of homoeosis might be applied to the phenomena of brachysm in cotton by considering that a partial translocation or homoeosis had taken place, for the characters of the internodes, leaves, and involucral bracts are intermediate. The leaves become more bractlike, the bracts more leaf-like. But to represent typical cases of homoeosis the leaves would need to be replaced by bracts or bracts by leaves, the

[1] "The difference between a tall and a dwarf pea is not the same as the difference between a tall and a dwarf man. In a human dwarf everything is on a smaller scale than in the normal man. But a dwarf pea is not simply a miniature edition, as it were, of a tall one—it differs from a tall pea in one single characteristic, the length of the internodes, i. e., the sections of the stem between two nodes, or joints, where the leaves are given off." Darbishire, A. D. Breeding and the Mendelian Discovery. p. 12–13. London, New York, 1911.

[2] Leavitt, R. G. A vegetative mutant, and the principle of homeosis in plants. *In* Bot. Gaz., v. 47, no. 1, p. 64. 1909.

primary idea being that of transference of characters from one place to another, which is not the same as abnormal or intermediate expression of characters. Leavitt's idea of homoeosis may appear to be in better accord with the Mendelian theory of heredity, which assumes that variations arise from differences in the transmission of characters, but metaphanic variations seem to represent differences in the expression of the characters rather than differences in transmission.

The present interpretation of metaphanic variations is based on the recognition of a definite distinction between transmission and expression as two essentially different processes, though usually described together under the general term "heredity." Characters are often transmitted without being expressed in visible form, as Darwin pointed out. Galton made a formal distinction between patent and latent characters—that is, between characters that are brought into expression and those that are transmitted without being expressed. Latency occurs frequently in connection with Mendelian characters and has received much attention in recent years, but the importance of the distinction between transmission and expression is often overlooked and theories of transmission are often applied to phenomena that belong to the field of expression. With this distinction in mind, the idea suggested by metaphanic variations like brachysm is not that the characters of one part have been transmitted in some irregular manner to another part of the plant, but that the normal sequence of changes or contrasts in the expression of the characters is no longer observed. It is reasonable to believe that all the characters are transmitted to all the parts, including those that usually do not serve the purposes of propagation. Each of the internodes of the cotton plant is capable of reproducing all the other parts. From this point of view the idea of translocation or transfer of characters no longer seems adequate to account for abnormal intermediate characters like brachysm. It seems more reasonable to think of metaphanic variations as arising because the characters are being confused or combined in expression.

In connection with the theory of homoeosis Leavitt suggested that the translocation of characters from one part of the body of a plant or animal to another part opened the way to evolutionary changes, and many examples were given in support of this interpretation. Intermediate expression of the characters, involving a loss of specialization or differentiation of parts, may also be considered as one of the ways in which plants may vary and thus initiate evolutionary changes, but reasons have been given already for looking upon metaphanic variations as negative or degenerative changes of characters rather than as having progressive evolutionary value. Instead of being taken as new characters that are being added to the mechanism of transmission, metaphanic variations may mean that the mechanism of expression has become deranged so that the old characters are not normally developed.

The prevalence of abortion in cluster cottons is one of the most striking evidences of the degenerative nature of such variations. In strongly clustered varieties completely sterile individuals are often found, the result of abortion of all the buds or young bolls or of all the buds which produce fruiting branches. Yet these completely sterile plants are less harmful to the stock than the other less abnormal degenerates that are able to reproduce themselves from seed and also to contaminate their neighbors with inferior pollen. That cluster cottons should seem more unstable than other varieties and more inclined to the production of abnormalities is easier to understand when they are considered as metaphanic variations, representing intermediate expressions of characters that are normally distinct.

AGRICULTURAL DEFECTS OF "CLUSTER" COTTONS

Having considered the general nature of the cluster character of cotton and the accompanying variations, we are in better position to understand the physiological status and practical value of such forms. The first impression of cluster varieties is that they are more fruitful, for they are able to set their buds and bolls more rapidly than varieties with normal fruiting branches. But when we have learned that brachysm is in the nature of a malformation and is frequently accompanied by malformations of leaves and bracts and abortion of both the floral and the vegetative buds, it becomes apparent that the brachytic variants are to be avoided by the breeder, especially when they bear other marks of degeneration.

The limited size and more upright habits of growth make it possible for more of the short-branched plants to stand in the same area, and very large yields may be obtained with favorable conditions of soil and season. But if the conditions prove unfavorable, cluster varieties are likely to suffer worse than others and to produce smaller crops, so that it is very doubtful whether the cluster habit is a practical advantage. The extreme forms of clustering are certainly undesirable, for in such varieties many of the plants are likely to become sterile through the blasting of all of the buds, the tendency to abortion being greatest when unfavorable conditions are encountered during the crop season.

Another agricultural consideration is that the crowding of the bolls together makes picking more difficult. This is especially true, of course, when clustering is accompanied by fasciation and other abnormalities, so that the bolls are malformed or misshapen. The opening of the bolls is also likely to be irregular, because cluster cottons often produce many late bolls on short branches developed from the axils of the leaves of the fruiting branches. Such bolls are usually undersized, as well as late in opening.

A further objection to the cluster character, especially in long-staple cottons, is that the lint of cluster varieties or of individual variations in

the direction of clustering is generally inferior to that of adjacent noncluster plants. This may be due to the fact already noted, the greater susceptibility of cluster plants to unfavorable conditions, owing to their restricted vegetative development. Occasional exceptions have been noticed, where cluster plants produced lint as good or better than that of noncluster neighbors, but there can be no doubt that the general tendency is in the direction of inferiority of lint.

CONCLUSIONS

Brachysm is a term proposed to designate the shortening of the vegetative internodes of plants. It is a hereditary abnormality, indicating degeneracy, that has appeared in independent mutative variations in many distinct families of plants, including many cultivated forms. Brachytic variations are of frequent occurrence in cotton, giving rise to the so-called "cluster" and "limbless" varieties, and afford unusually favorable opportunities for learning the nature and physiological significance of such variations.

The shortening of the internodes of the cotton plant is usually confined to the fruiting branches without affecting the main stalk or the vegetative branches. Brachytic variations occur independently in different species and varieties of cotton and do not constitute a natural group with a common origin.

Brachytic varieties of cotton usually show other abnormalities of the internodes, leaves, and involucral bracts. There is also an increased tendency to abortion of the floral buds, and the blasted buds often remain attached to the plant, because of the absence of well-differentiated absciss-layer at the base of the pedicel.

Though brachytic variations arise by mutative changes in the expression of the characters and show alternative Mendelian forms of inheritance, they afford no additional support to the general theories of mutation and Mendelism as explaining evolution. Such variations represent reduced specialization or intermediate expression of characters and are degenerative in nature. They are not to be considered as examples of normal heredity or of the evolution of new characters. The abnormalities of brachytic variations are analogous to those found among hybrids and are likewise accompanied by tendencies to sterility or abortion of buds.

Brachysm is to be associated with other forms of intermediate expression of characters, representing a general class of metaphanic variations. A more definite recognition of this class of variations is desirable in connection with the investigation of general problems of heredity and evolution.

The agricultural value of brachytic varieties of cotton is impaired by the tendency to abnormal variations and sterility and also by the fact that the cluster cottons are more severely affected by unfavorable conditions. Hence, brachysm is to be avoided in the breeding of superior varieties of cotton.

PLATE LIII

Abnormal simple leaf on fruiting branch of Egyptian cotton, accompanied by abnormal leaf-like bract, remainder of involucre and floral bud removed. Natural size. Photographed by Mr. G. B. Gilbert.

PLATE LIV

Normal 3-lobed leaf of fruiting branch of Egyptian cotton, accompanied by normal involucral bract for comparison with Plate LIII. Natural size. Photographed by Mr. G. B. Gilbert.

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PLATE LV

Abnormal leaf of fruiting branch of Egyptian cotton with one stipule enlarged and the lobe of the same side wanting. Natural size. Photographed by Mr. G. B. Gilbert. Specimen from Bard, Cal., on October 2, 1913.

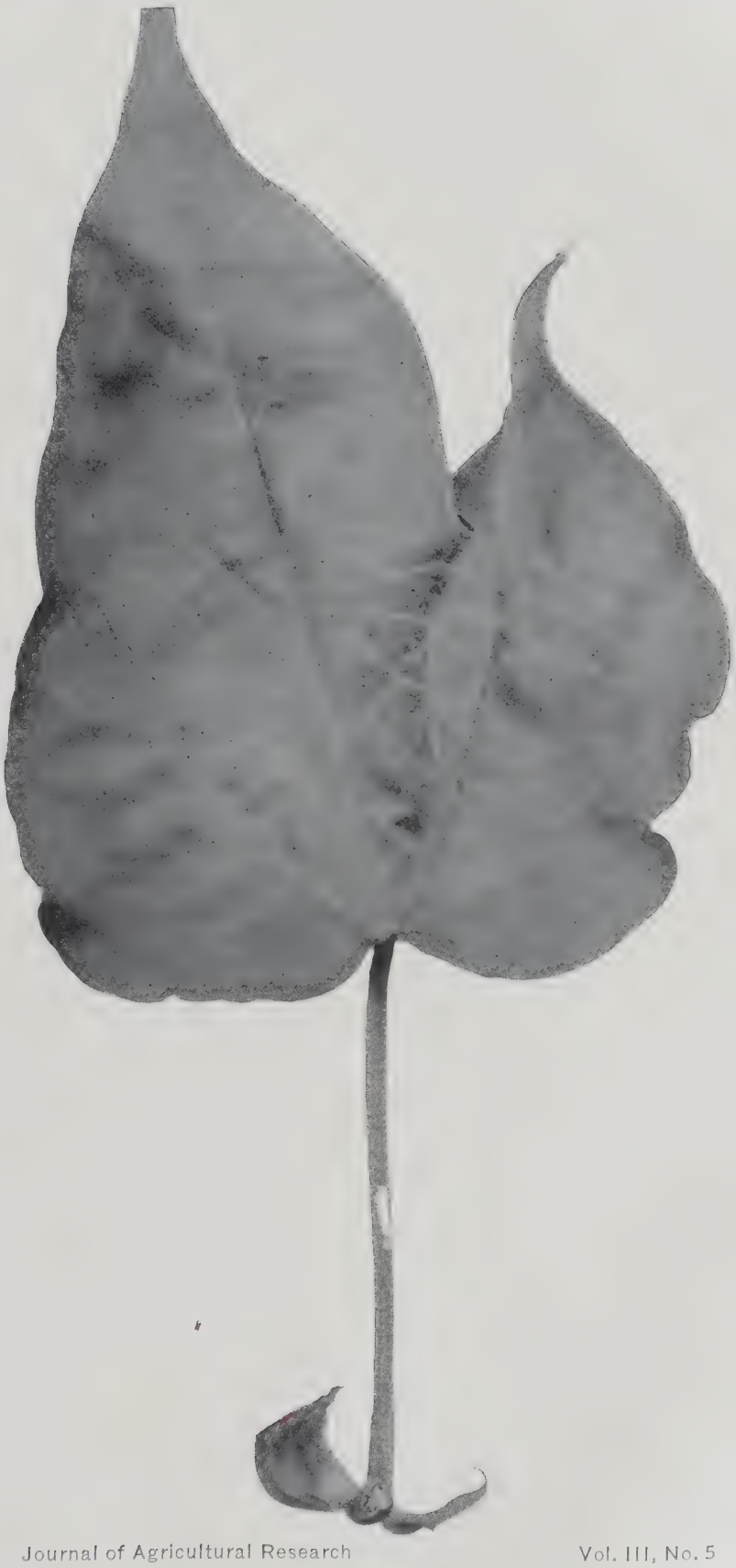

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PLATE LVI

Brachytic fruiting branches of "cluster" cotton (Willets Red Leaf) shortened to a single internode by abortion of terminal bud. Natural size. Photographed by Mr. C. B. Doyle.

Fig. 1.—The boll at the right is borne by a very short branch from an axillary bud.

Fig. 2.—The boll at the right is borne by the shortened fruiting branch. The left-hand boll represents a shortened branch in the axil of the leaf that subtends the fruiting branch.

PLATE LVII

Normal and brachytic joints on same fruiting branch of Upland cotton. Natural size. Photographed by Mr. C. B. Doyle. Specimen from Bard, Cal., on October 13, 1912.

PLATE LVIII

Branches of abnormal variation of Upland cotton, with abortive buds remaining attached to branches by decurrent pedicels and elongated bud scars. The left-hand branch shows abnormal inequality in the lengths of the internodes.

PLATE LIX

Portion of brachytic fruiting branch of Simpkins cotton producing twin fasciated branches from an axillary bud. The boll of the next internode has an elongated, somewhat decurrent base, while that of the third internode of the branch is aborted and shriveled, only the pedicel being shown, at the left of the plate. Natural size. Photographed by Mr. C. B. Doyle.

PLATE LX

Portion of fruiting branch of Columbia cotton, with one internode adnate to the pedicel of the boll of the preceding internode. Specimen from Easley, S. C. Natural size. Photographed by Mr. C. B. Doyle.

PLATE LXI

Fig. 1.—Plant of Dale Egyptian cotton, showing complete abortion of fruiting branches on the main stalk, while the vegetative branches of the same plant produced a few fruiting branches and ripened a few bolls. Plant grown at Bard, Cal. Photographed by Mr. C. B. Doyle.

Fig. 2.—End of main stalk of plant shown in figure 1, showing abortion of terminal bud and compensatory thickening of the petioles. Natural size. Photographed by Mr. C. B. Doyle.

2

PLATE LXII

Ends of main stalks of two plants of Dale Egyptian cotton, showing simple fruiting branches and closely similar axillary fruiting branches. In one case the terminal bud was aborted, while in the other it was transformed into a boll subtended by an abnormal bract-like leaf with the stipules enlarged and the blade entire, instead of 5-lobed. Plants grown at Bard, Cal. Photographed by Mr. C. B. Doyle.

ABILITY OF COLON BACILLI TO SURVIVE PASTEURIZATION

By S. HENRY AYERS, *Bacteriologist*, and W. T. JOHNSON, Jr., *Scientific Assistant*, *Dairy Division, Bureau of Animal Industry*

INTRODUCTION

The presence of colon bacilli in pasteurized milk is generally interpreted as meaning either that the milk was not properly heated or that it was reinfected after pasteurization by careless handling. This interpretation is based on the low thermal death point of cultures of *Bacillus coli*.

Van Geuns (4)[1] in 1899 found that the *Bacillus neapolitanus* of Emmerich, the same organism as the *B. coli communis* of Escherich, was destroyed by heating for five minutes at 59° C. (138.2° F.) and one minute at 62.5° C. (144.5° F.). Based on the work of Van Geuns, Ringeling (6) examined 75 samples of pasteurized milk from 24 dairies in Amsterdam for the presence of colon bacilli. In 16 per cent of the samples examined he found *B. coli* present. Since colon bacilli were found in pasteurized milk from 10 of the 24 dairies, Ringeling concluded that this proportion (41 per cent) of the dairies in Amsterdam did not pasteurize or handle the milk properly.

During recent years numerous investigators have studied cultures of *B. coli* and found that the organisms were easily destroyed at temperatures below 60° C. (140° F.), which is the lowest pasteurizing temperature.

In a previous study by the writers (1) of the bacteria which survive pasteurization 19 samples of raw milk in sterile flasks were heated for 30 minutes at 62.8° C. (145° F.). Each sample after pasteurization was examined carefully for the presence of colon bacilli, but none were found.

All these results naturally strengthened the opinion that the presence of *B. coli* in pasteurized milk might be a valuable index as to the efficiency of the process and the care observed in handling the milk after the heating process.

At times, however, it has been found that high temperatures were required to destroy cultures of *B. coli*. Gage and Stoughton (3) in a study of the resistance of *B. coli* to heat found that sometimes cultures were destroyed only by heating to temperatures much higher than the usual thermal death point. Heat-resistant strains have been also found by De Jong and De Graaff (5). Certain strains with which they worked re-

[1] Reference to "Literature cited" is made by number, p. 409.

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quired 30 minutes heating at 70°–72° C. (158° to 161.6° F.), in order to destroy them. Zelenski (9) also found strains of *B. coli* which resisted high temperatures.

In view of this uncertainty regarding the thermal death point of *B. coli* and its relation to pasteurizing temperatures, the following experimental work concerning the ability of colon bacilli to survive pasteurization was undertaken. The number of cultures used in the experiments was 174.

METHOD OF DETERMINING THE THERMAL DEATH POINT

The colon cultures were grown first in plain, neutral extract broth for 18 hours and then inoculated by means of a small-bore pipette into litmus-milk tubes. Four drops constituted an inoculation in each milk tube. In making the inoculations care was taken not to have any of the culture touch or any of the inoculated milk wash upon the sides of the tube, either during the handling or during the subsequent heating.

The inoculated milk tubes, with the exception of the control tubes, were heated in a water bath in which the water was agitated, and the temperature of the milk was recorded in a control tube by a thermometer placed in the milk. The temperature in the tubes was not allowed to vary more than half a degree in either direction. In all experiments the heating period was 30 minutes at a given temperature. After the heating, the tubes of milk were quickly cooled to about 10° C. (50° F.), incubated at 37° C. (98.6° F.), and the reactions recorded after 24, 48, 72, and 96 hours. Growth in the tube indicated that the organism was not destroyed at the particular temperature to which the milk had been subjected. In every case the tubes were run in duplicate, and in general both tubes had to show growth before the test was considered positive. The only exceptions to this were cases in which only one of the tubes showed growth after the highest heating temperature; in such cases one tube was considered a positive reaction and the organism was recorded as surviving the process.

This method of determining the thermal death point was used, in order to render the conditions of heating similar to pasteurization.

THE THERMAL DEATH POINT OF THE CULTURES AS A WHOLE

Studies were made of the thermal death point of 174 cultures of colon bacilli isolated from the following sources: 154 from cow feces, 16 from milk and cream, 2 from flies, 1 from human feces, and 1 from cheese. (The cultures were supplied through the courtesy of Mr. L. A. Rogers, in charge of the research laboratory of the Dairy Division.) All of the organisms would be classified as colon bacilli according to the usual cultural tests for *Bacillus coli*. These cultures, with the exception of two not studied and three noted below, were typical colon bacilli of the

B. coli communis or *communior* type according to the studies of Rogers, Clark, and Davis (7), also Rogers, Clark, and Evans (8). The three cultures not typical were probably of the *B. aerogenes* type. The cultures were heated in milk as previously described to temperatures ranging from 51.7° C. (125° F.) to 68.3° C. (155° F.). The results given in Table I show the number and percentage of cultures which withstood the different temperatures.

TABLE I.—*Effect of heat on colon bacilli—all cultures*

Cultures surviving.	Exposed for 30 minutes at—						
	51.7° C. (125° F.).	54.5° C. (130° F.).	57.2° C. (135° F.).	60° C. (140° F.).	62.8° C. (145° F.).	65.6° C. (150° F.).	68.3° C. (155° F.).
Number	174	173	158	95	12	1	0
Per cent	100. 00	99. 43	90. 80	54. 59	6. 89	0. 57	0

It is seen from the table that 95, or 54.59 per cent of all the cultures, survived at 60° C. (140° F.). This is particularly interesting, since this temperature is the lowest used in commercial pasteurization. When heated to 62.8° C. (145° F.), 12, or 6.89 per cent, of the cultures survived. This temperature of 62.8° C. (145° F.) maintained for 30 minutes is the temperature generally used in the process of pasteurization. Only one culture survived a temperature of 65.6° C. (150° F.), and this culture when heated again failed to survive at this temperature.

These results are shown more clearly in figure 1, where they have been plotted. One of the cultures was destroyed at a temperature as low as 54.5° C. (130° F.). It is interesting to note that at 60° C. (140° F.) 95 of the cultures survived, while at 62.8° C. (145° F.) a difference of only 2.8° C. or 5° F., only 12 survived. In other words, 87.3 per cent of the cultures which survived at 60° C. (140° F.) were destroyed at 62.8° C. (145° F.).

It is very evident from these results that colon bacilli may survive the process of pasteurization when a temperature of 62.8° C. (145° F.) is used.

VARIATION IN THE THERMAL DEATH POINT OF THE CULTURES

In order to determine whether the colon bacilli which survive at 62.8° C. (145° F.) would exhibit the same ability in repeated heatings, the same cultures were reheated to that temperature six times, with the results shown in Table II.

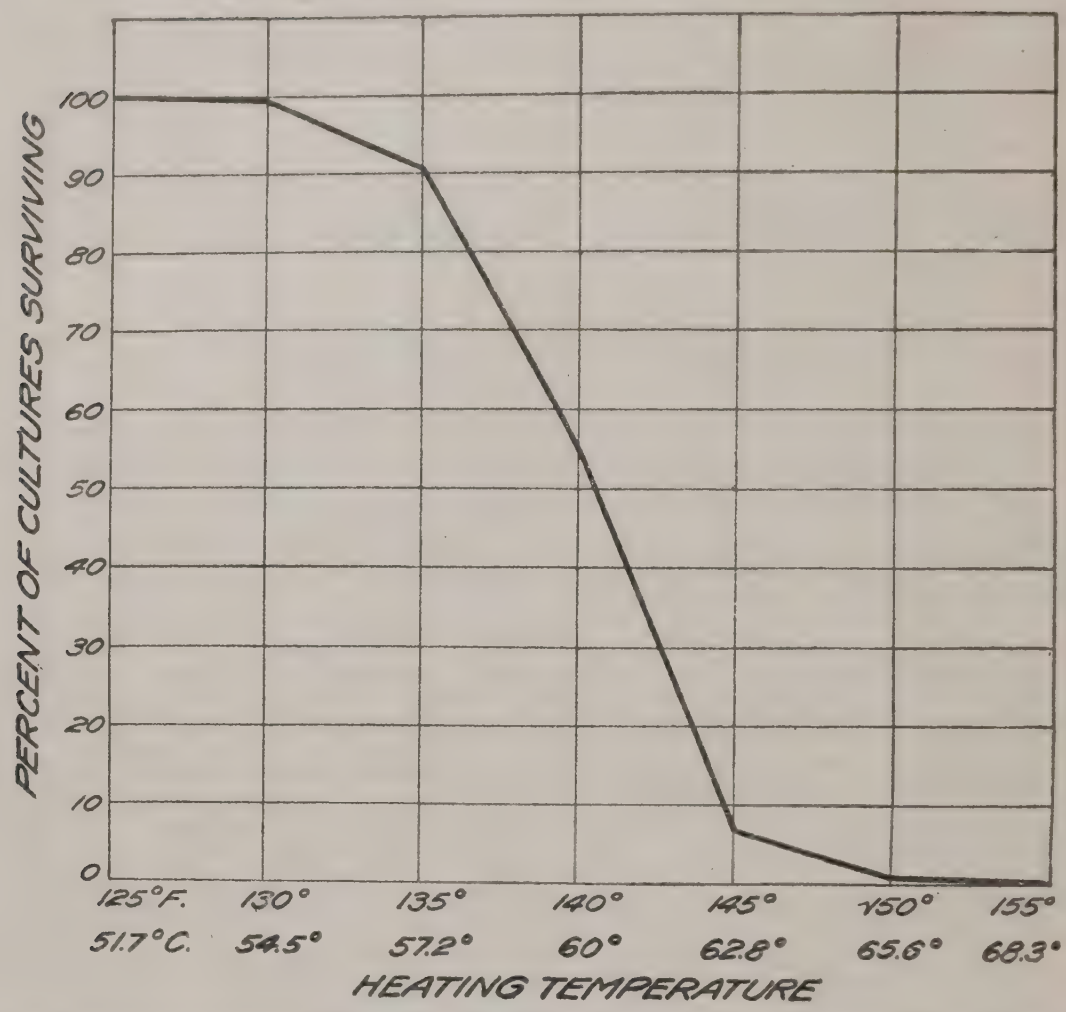

FIG. 1.—Curve showing results of heating cultures of colon bacilli for 30 minutes at various temperatures.

TABLE II.—*Variation in the ability of colon bacilli to survive pasteurization for 30 minutes at 62.8° C. (145° F.)*

Culture No.	Number of heating.						Summary.		
	First.	Second.	Third.	Fourth.	Fifth.	Sixth.	Both tubes +.	Both tubes −.	One tube + and one tube −.
GV	++	+−	−−	++	++	−−	3	2	1
HO	+−	−−	+−	−−	+−	−−	0	0	3
HP	+−	+−	++	++	−−	−−	2	2	2
IF	+−	−−	+−	−−	+−	−−	0	3	3
IJ	+−	−−	−−	−−	+−	−−	0	4	2
IL	+−	+−	++	+−	−−	−−	1	2	3
IN	+−	−−	−−	+−	+−	−−	0	3	3
IS	+−	−−	+−	−−	++	−−	1	3	2
IY	+−	−−	+−	+−	+−	−−	0	2	4
MF	+−	−−	−−	−−	+−	−−	0	4	2
MT	+−	−−	+−	++	+−	−−	1	2	3
NV	+−	+−	+−	−−	−−	−−	0	3	3
Total cultures	12	12	12	12	12	12	.....	.....	.....
Positive	12	4	8	6	9	0	.....	.....	.....

In the above experiments two litmus-milk tubes were inoculated in the usual way with each of the 12 cultures and were heated for 30 minutes at 62.8° C. (145° F.). The first experiment shows the record of the 12 cultures which survived in the determination of the thermal death point of the cultures as a whole. When these 12 cultures were again heated, only 4 survived, on the third trial 8, on the fourth trial 6, on the fifth trial 9, and on the sixth trial none survived.

These results are important since they show that at 62.8° C. (145° F.) colon bacilli may or may not survive a process of pasteurization. It is evident that this is a critical temperature and that occasionally colon bacilli may survive, owing in all probability to the resistance of a few cells in the culture. This explanation is supported by the figures in the summary of Table II, in which is seen the number of times that both the litmus-milk tubes showed growth; also when both tubes were negative and when one tube was positive and one negative.

It may be seen, also, that the same culture on repeated heating does not give the same results. It is evident that certain strains of colon bacilli have a thermal death point which is close to 62.8° C. (145° F.), and although they represent only a small percentage of the cultures we studied, the fact that such cultures exist complicates the colon test for efficiency of pasteurization.

The apparent scarcity of these resistant colon bacilli and the fact that 62.8° C. (145° F.) is near their thermal death point explains our failure to find them in the samples pasteurized by us under laboratory conditions, as stated previously in this paper.

HEAT RESISTANCE OF COLON BACILLI

From these results it seems that the colon bacilli as a rule have a low majority thermal death point and the cultures survive the higher temperatures only by reason of the resistance of a few cells.

Gage and Stoughton (3) found this to be true of a few cultures which they studied, and although they tried to breed a race with a high majority thermal death point, their efforts were not successful. We have also tried to breed a resistant type, but thus far without success.

In our experiments the thermal death point determinations were made in duplicate tubes of litmus milk, and the appearance of growth was recorded after an incubation period of 24, 48, 72, and 96 hours. In every case control tubes not heated showed a marked acid reaction in 24 hours, but in the heated tubes with the same inoculation the growth was often delayed, so that sometimes no reaction was noticed until after 96 hours, incubation. When the reaction was delayed, it showed that a portion of the bacteria in the milk were destroyed by the heating. When the heating has little or no effect, the heated tubes should show a positive reaction

in 24 hours the same as the control tubes. In Table III we have recorded the percentage of cultures which gave a positive reaction in both of the litmus-milk tubes and in only one milk tube after different periods of incubation and when heated at different temperatures.

TABLE III.—*Effect of heat in relation to the time required for cultures to show growth*

Hours of incubation.	Tube reaction.	54.5° C. (130° F.).	57.2° C. (135° F.).	60° C. (140° F.).	62.8° C. (145° F.).
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
24	2 tubes+	92. 75	28. 34	11. 11	12. 50
	1 tube+, 1 tube−	0	14. 17	26. 98	0
48	2 tubes+	5. 80	24. 41	17. 46	0
	1 tube+, 1 tube−	1. 45	12. 59	20. 63	62. 50
72	2 tubes+	0	5. 52	3. 17	0
	1 tube+, 1 tube−	0	11. 03	19. 06	25. 00
96	2 tubes+	0	0	0	0
	1 tube+, 1 tube−	0	3. 94	1. 59	0
Total		100. 00	100. 00	100. 00	100. 00

From the table it will be seen that 92.75 per cent of the cultures showed a positive reaction after 24 hours' incubation in both of the duplicate tubes when heated at 54.5° C. (130° F.). After 48 hours' incubation 5.8 per cent more of the cultures showed a positive reaction in both tubes, while 1.45 per cent showed a positive reaction in only one of the two tubes. At 62.8° C. (145° F.), however, only a small percentage of the cultures were positive after 24 hours. The majority required from 48 to 72 hours' incubation to show growth. This shows that a large proportion of the cells were destroyed so that a longer incubation was necessary to allow bacterial increases sufficient to cause a positive reaction. These facts are further supported by the differences in the number of cultures in which both duplicate tubes showed a positive reaction. At 62.8° C. (145° F.) only a small percentage of the cultures showed a positive reaction in both tubes, showing that in many cases all the bacteria in one tube were destroyed, while in the duplicate tube a few cells only survived.

It is of interest to note that the colon bacilli are less heat-resistant than the streptococci, as is shown in a previous paper (2).

THE PRESENCE OF COLON BACILLI AS A TEST OF THE EFFICIENCY OF PASTEURIZATION

The growth of colon bacilli which survive pasteurization is a matter of considerable importance, particularly when the presence of *B. coli* in pasteurized milk is considered as an index of the efficiency of the process. We have therefore studied the effect of pasteurization at 62.8° C. (145° F.) on two cultures of colon bacilli which were known to be able to survive heating at that temperature. Flasks of sterile skim milk were inoculated with several cubic centimeters of an 18-hour-old broth culture of a colon bacillus. The number of bacteria in the milk was determined before heating and again after the pasteurized milk had been allowed to stand for 24, 48, and 72 hours at room temperature. The bacteria at the end of the 24-hour period were determined by placing as high as 3 cubic centimeters in large petri plates. Table IV shows the results of an experiment with the two colon cultures, GV and HO. Three flasks of milk were inoculated from each culture with the same amount of broth, but a bacterial count was made on only one of the three flasks before heating.

TABLE IV.—*Growth of Bacillus coli in milk heated for 30 minutes at 62.8° C. (145° F.) and held at room temperature*

Culture No.	Flask.	Number of bacteria per cubic centimeter.			
		Before pasteurization.	After pasteurization.		
			24 hours.	48 hours.	72 hours.
GV	1	5,000,000	0 in 3 c. c.	1,050,000	20,000,000
	2		1 in 3 c. c.	20,000	1,140,000,000
	3		0 in 3 c. c.	210,000,000	1,450,000,000
HO	1	6,000,000	0 in 3 c. c.	61,000,000	1,750,000,000
	2		0 in 2 c. c.	172,000,000	1,250,000,000
	3		1 in 3 c. c.	160,000,000	800,000,000

It may be seen from the table that, although the cultures survived the pasteurization at 62.8° C. (145° F.), there was a very great cell destruction, as the bacterial count after the flasks had stood for 24 hours at room temperature was very low. However, in 48 hours' time there was a very large bacterial increase and even more after 72 hours.

A similar experiment was repeated with the same cultures, except that the milk after heating was held in a refrigerator at 8 C. (46.4° F.). The results in Table V show again the great destruction of bacterial cells which takes place during the heating process. A few bacteria survived, but very little increase took place at the low temperature.

TABLE V.—*Growth of Bacillus coli in milk heated for 30 minutes at 62.8° C. (145° F.) and held at 8° C. (46.4° F.)*

Culture No.	Flask.	Number of bacteria per cubic centimeter.							
		Before pasteurization.	After pasteurization.						
			1 day.	2 days.	3 days.	4th–9th day.	3 weeks.	4 weeks.	6 weeks.
GV.....	1	4,000,000	0 in 3 c. c.	0 in 2 c. c.	0 in 3 c. c.	0 in 3 c. c.	50	10	0 in 3 c. c.
HO.....	1	7,000,000	1 in 3 c. c.	0 in 2 c. c.	0 in 3 c. c.	0 in 3 c. c.	1,000	60	0 in 3 c. c.
	2		1 in 3 c. c.	0 in 3 c. c.	0 in 2 c. c.	0 in 3 c. c.	500	70	0 in 3 c. c.

The relation of these results to commercial pasteurization can be plainly seen. Milk is pasteurized usually at 62.8° C. (145° F.) for 30 minutes and in subsequent handling is kept at various temperatures from low to high, depending on conditions, until it is consumed. In view, therefore, of the results of our experiments, it is possible to explain the presence of colon bacilli in pasteurized milk on the ground of their ability to survive the process.

These results, however, indicate that colon bacilli survive pasteurization on account of the resistance of a few cells and not because the cultures have a high majority thermal death point, in which case a large number of cells would survive. Since it is apparent that colon bacilli have a low majority thermal death point, we should not expect to find large numbers of these bacteria in pasteurized milk immediately after the heating process. If this condition is found we should believe from our results that the presence of the bacilli would indicate inefficient heating or a heavy reinfection.

We must call attention to the fact that these opinions are based on a study of 174 cultures of colon bacilli, and consequently, while they represent a considerable number of strains of *Bacillus coli*, it is possible that a study of still more cultures might yield different results. It is not improbable that colon bacilli with a high majority thermal death point do exist, and if such is the case large numbers might be found immediately after pasteurization.

SUMMARY AND CONCLUSIONS

(1) The thermal death point of 174 cultures of colon bacilli isolated from cow feces, milk and cream, human feces, flies, and cheese showed considerable variation when the cultures were heated in milk for 30 minutes under conditions similar to pasteurization.

At 60° C. (140° F.), the lowest pasteurizing temperature, 95 cultures, or 54.59 per cent, survived; at 62.8° C. (145° F.), the usual temperature for pasteurizing, 12, or 6.89 per cent, survived. One culture was not destroyed at 65.6° C. (150° F.) on the first heating, but in repeated experiments it was always destroyed.

(2) There is a marked difference in the effect of heating at 60° C. (140° F.) and at 62.8° C. (145° F.). Although there is only a difference of 2.8° C., or 5° F., 87.3 per cent of the cultures which survived at 60° C. (140° F.) were destroyed at 62.8° C. (145° F.).

(3) Considerable variation was found in the thermal death point of the colon bacilli which survived 62.8° C. (145° F.). When the 12 cultures which survived were heated again at the same temperature, it was found that many did not survive and in each repeated heating different results were obtained.

It seems evident that 62.8° C. (145° F.) maintained for 30 minutes is a critical temperature for colon bacilli.

(4) Among the 174 cultures studied all were found to have a low majority thermal death point, but were able to survive pasteurizing temperatures on account of the survival of a few cells.

(5) The colon test as an index of the efficiency of the process of pasteurization is complicated by the ability of certain strains to survive a temperature of 62.8° C. (145° F.) for 30 minutes and to develop rapidly when the pasteurized milk is held under temperature conditions which might be met during storage and delivery.

The presence of a large number of colon bacilli immediately after the heating process may indicate improper treatment of the milk.

(6) If milk is pasteurized at a temperature of 65.6° C. (150° F.) or above for 30 minutes, we should not expect, from our results, that any colon bacilli would survive. Consequently under such conditions the colon test for the efficiency of pasteurization may be of value. It must be remembered, however, that a study of more cultures may reveal strains of colon bacilli that are able to survive this and even higher temperatures.

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PRELIMINARY AND MINOR PAPERS

FITTING LOGARITHMIC CURVES BY THE METHOD OF MOMENTS [1]

By JOHN RICE MINER,

Computer, Maine Agricultural Experiment Station

WITH AN INTRODUCTORY STATEMENT ON THE USE OF LOGARITHMIC CURVES IN BIOLOGICAL AND AGRICULTURAL INVESTIGATIONS BY RAYMOND PEARL, BIOLOGIST, MAINE AGRICULTURAL EXPERIMENT STATION

INTRODUCTORY STATEMENT

The use of logarithmic curves in the analysis of various kinds of biological and agricultural data is rapidly becoming widespread and general. It was first shown by Lewenz and Pearson (13, 22) [2] that the growth of children followed a logarithmic curve. The present writer (17) demonstrated that the phenomena of growth and differentiation in Ceratophyllum also followed a logarithmic curve. Donaldson (2, 3, 4, 5, 6) and Hatai (8, 9, 10) in a series of papers dealing with the growth and quantitative relations of the whole organism and its various parts in the white rat and the frog have shown that the same law holds for growth in those forms.

Other biological phenomena than growth follow a logarithmic law. Pearl (14), in a case of regulation of the shape of abnormal eggs, and later Curtis (1) for normal eggs, have shown that the changes in size and shape of successively laid eggs are graduated with a logarithmic curve. Work now in progress in the Biological Laboratory, Maine Experiment Station, of which only a preliminary notice has yet been published (15), shows that generally the change in milk flow with age in dairy cattle is logarithmic. Several years ago Holtsmark (12) pointed out that the relation between the number of food units required and the milk yields of different animals was logarithmic.

From this incomplete review of the literature recording the use of logarithmic curves in biological and agricultural investigations it is clear that the workers in these fields will, as time goes on, have increasing need to be able to handle these curves easily and critically.

Up to the present time the only available method of fitting logarithmic curves was that of least squares. Several years ago Pearl and McPheters (16) published a set of tables intended to lighten materially the labor of fitting such curves by the least-squares method. For a long time, however, the writer has felt that it would be highly desirable to bring this class of curves into the general system of curve fitting worked out by Pearson (18, 19, 20, 21, 23), and known as the "method of moments." The theory of the method is extremely simple, involving as

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 78.

[2] Reference is made by number to "Literature cited," p. 422.

it does only the assumption that if we equate the area and moments of a theoretical curve to the area and moments of a series of observations we shall get a reasonable fit of the curve to the observations. Experience with the method in the hands of different workers in England and America has abundantly demonstrated that this assumption is entirely justified in the fact.

In the papers cited, and in others also, Pearson has given the equations for the calculation of the constants from the moments in the case of (*a*) skew frequency curves in general, (*b*) sine curves, (*c*) parabolas of all orders, (*d*) the point binomial, (*e*) hypergeometrical series, etc. There has been lacking, however, the determination of the equations connecting moments and constants for the general family of logarithmic curves of the type

$$y=a+bx+cx^2+d\log(x+\alpha)$$

and its modifications. I suggested some time ago to Mr. Miner that he attack the problem, which, while theoretically simple and straightforward, proved rather laborious in the actual carrying out. This he has done, with the results set forth in this paper.

RAYMOND PEARL

MOMENTS OF A LOGARITHMIC CURVE

GENERAL CASE

Let $y_1, y_2, y_3, \ldots, y_k, \ldots, y_m$ be a series of ordinates with corresponding abscissæ $x_1, x_2, x_3, \ldots, x_k, \ldots x_m$ to which is to be fitted the curve $y=a+bx+c\log_{10}x$.

Let the unit of calculation $=x_2-x_1$ and the origin be placed at $2x_1-x_2$. The abscissæ expressed in units of calculation and taken from the new origin will then be $1, 2, x'_3, \ldots, x'_k, \ldots x'_m$. In calculating the moments each ordinate must first be multiplied by the base, $h_{x'_k}$, of the rectangle of which it is the mid line. Otherwise the moments will represent not the whole area, but strips of unit base of which the ordinates are the mid lines. For the first three ordinates $h_{x_1 k}$ is 1, 1, $2x'_3-5$, respectively; for higher ordinates it is found from the equation $h_{x'_k}=2x'_k-4x'_{k-1}+4x'_{k-2}-\ldots-(-1)^k\,4x'_3+(-1)^k\,5$. The upper limit of the area is given by $2x'_m-2x'_{m-1}+\ldots-(-1)^k\,2x'_3+(-1)^k\,\frac{5}{2}=l+q$, where l is the integral and q the fractional portion of the number.

Let M_n represent the nth moment about the origin as above chosen. Then

$$M_n=\int_{\frac{1}{2}}^{l+q}(a+bx+c\,\log_{10}x)\;x^n dx=\frac{a}{n+1}\left[(l+q)^{n+1}-\left(\frac{1}{2}\right)^{n+1}\right]$$

$$+\frac{b}{n+2}\left[(l+q)^{n+2}-\left(\frac{1}{2}\right)^{n+2}\right] \qquad \text{(i)}$$

$$+c\,\log_{10}e\left[(l+q)^{n+1}\left\{\frac{\log_e(l+q)}{n+1}-\frac{1}{(n+1)^2}\right\}-\left(\frac{1}{2}\right)^{n+1}\left\{\frac{\log_e\frac{1}{2}}{n+1}-\frac{1}{(n+1)^2}\right\}\right]$$

Putting $n=0, 1, 2$, successively, we obtain the three equations which being solved give us the constants of the curve:

$$M_0=a\left(l+q-\tfrac{1}{2}\right)+\frac{b}{2}\left[(l+q)^2-\tfrac{1}{4}\right]+c\log_{10}e\left[(l+q)\log_e(l+q)-\tfrac{1}{2}\log_e\tfrac{1}{2}+\tfrac{1}{2}-(l+q)\right] \quad \text{(ii)}$$

$$M_1=\frac{a}{2}\left[(l+q)^2-\tfrac{1}{4}\right]+\frac{b}{3}\left[(l+q)^3-\tfrac{1}{8}\right]+\frac{c}{2}\log_{10}e\left[(l+q)^2\log_e(l+q)-\tfrac{1}{4}\log_e\tfrac{1}{2}+\tfrac{1}{8}-\tfrac{1}{2}(l+q)^2\right] \quad \text{(iii)}$$

$$M_2=\frac{a}{3}\left[(l+q)^3-\tfrac{1}{8}\right]+\frac{b}{4}\left[(l+q)^4-\tfrac{1}{16}\right]+\frac{c}{3}\log_{10}e\left[(l+q)^3\log_e(l+q)-\tfrac{1}{8}\log_e\tfrac{1}{2}+\tfrac{1}{24}-\tfrac{1}{3}(l+q)^3\right] \quad \text{(iv)}$$

Multiplying equation ii by $\frac{1}{2}\left(l+q+\frac{1}{2}\right)$ and by $\frac{1}{3}\left[(l+q)^2+\frac{1}{2}(l+q)+\frac{1}{4}\right]$ and subtracting from equation iii and iv, respectively:

$$2M_1-\left(l+q+\tfrac{1}{2}\right)M_0=\frac{b}{6}\left(l+q-\tfrac{1}{2}\right)^3+\frac{c}{2}\log_{10}e\left[(l+q)\left\{\log_e\tfrac{1}{2}-\log_e(l+q)\right\}+(l+q)^2-\tfrac{1}{4}\right] \quad \text{(v)}$$

$$3M_2-\left[(l+q)^2+\tfrac{1}{2}(l+q)+\tfrac{1}{4}\right]M_0=\frac{b}{4}\left(l+q+\tfrac{1}{2}\right)\left(l+q-\tfrac{1}{2}\right)^3+\frac{c}{2}\log_{10}e\left[(l+q)\left(l+q+\tfrac{1}{2}\right)\left\{\log_e\tfrac{1}{2}-\log_e(l+q)\right\}+\tfrac{4}{3}(l+q)^3-\tfrac{1}{6}\right] \quad \text{(vi)}$$

Multiplying equation v by $\frac{3}{2}\left(l+q+\frac{1}{2}\right)$ and subtracting from equation vi:

$$6\left[M_2-\left(l+q+\tfrac{1}{2}\right)M_1\right]+\left[(l+q)^2+2(l+q)+\tfrac{1}{4}\right]M_0=\frac{c}{2}\log_{10}e\left[(l+q)\left(l+q+\tfrac{1}{2}\right)\left\{\log_e(l+q)-\log_e\tfrac{1}{2}\right\}-\tfrac{1}{3}\left\{(l+q)^3+\tfrac{9}{2}(l+q)^2-\tfrac{9}{4}(l+q)-\tfrac{1}{8}\right\}\right] \quad \text{(vii)}$$

$$c=\frac{6\left[\left(l+q+\tfrac{1}{2}\right)M_1-M_2\right]-\left[(l+q)^2+2(l+q)+\tfrac{1}{4}\right]M_0}{\frac{1}{2}\left[(l+q)\left(l+q+\tfrac{1}{2}\right)\left\{\log_{10}\tfrac{1}{2}-\log_{10}(l+q)\right\}+\tfrac{1}{3}\left(l+q-\tfrac{1}{2}\right)\left\{(l+q)^2+5(l+q)+\tfrac{1}{4}\right\}\log_{10}e\right]} \quad \text{(viii)}$$

$$b=\frac{6}{\left(l+q-\frac{1}{2}\right)^3}\left[2M_1-\left(l+q+\frac{1}{2}\right)M_0 - \frac{c}{2}\left\{(l+q)\left(\log_{10}\frac{1}{2}-\log_{10}l+q\right)+\left(\overline{l+q}^2-\frac{1}{4}\right)\log_{10}e\right\}\right] \quad \text{(ix)}$$

$$a=\frac{1}{l+q-\frac{1}{2}}\left[M_0-\frac{b}{2}\left\{(l+q)^2-\frac{1}{4}\right\}-c\left\{(l+q)\log_{10}(l+q) -\frac{1}{2}\log_{10}\frac{1}{2}-\left(l+q-\frac{1}{2}\right)\log_{10}e\right\}\right] \quad \text{(x)}$$

SPECIAL CASES

In the preceding section the simplest form of logarithmic curve in practical biometric use is considered. Extended experience in the Biological Laboratory of the Maine Experiment Station has shown that this simple form of the curve is only rarely adequate in the fitting of biological data. Usually one or the other, or both, of two modifications is found to be necessary before a suitable logarithmic curve is found. One of these, first used in biometric work by Pearl (17), in his studies of the growth of Ceratophyllum, later by Hatai (8) and others, is to add another constant, α, so that the equation then becomes

$$y=a+bx+c\log(x+\alpha).$$

The second modification is made by adding a term in x^2 to the equation. This modification is necessary in the wide range of cases where, after reaching a maximum, the values of the ordinates decrease with increasing values of x. This curve with the x^2 term was first used by Pearl to describe the change in shape of successively laid eggs of a particular hen (14). A logarithmic curve of this type

$$y=a+bx+cx^2+d\log x$$

appears, from rather extensive experience in this laboratory, to be the general form of expression of the quantitative changes in an organism throughout its life—that is, *including both growth and senescence*.

The equations for determining the constants of the curve

$$y=a+bx+cx^2+d\log_{10}x$$

are as follows:

$$d=\frac{\left\{\begin{array}{c}20M_3-30\left(l+q+\frac{1}{2}\right)M_2+3[4(l+q)^2+6(l+q)+1]M_1 \\ -\left(l+q+\frac{1}{2}\right)\left[(l+q)^2+4(l+q)+\frac{1}{4}\right]M_0\end{array}\right\}}{\left\{\begin{array}{l}\frac{1}{2}\left[(l+q)\left\{(l+q)^2+\frac{3}{2}(l+q)+\frac{1}{4}\right\}\left\{\log_{10}\frac{1}{2}-\log_{10}(l+q)\right\}\right] \\ +\frac{1}{6}\left\{(l+q)^2-\frac{1}{4}\right\}\left\{(l+q)^2+14(l+q)+\frac{1}{4}\right\}\log_{10}e\end{array}\right\}} \quad \text{(xi)}$$

$$c=\frac{30}{\left(l+q-\frac{1}{2}\right)^5}\left[6\left\{M_2-\left(l+q+\frac{1}{2}\right)M_1\right\}+\left\{(l+q)^2+2(l+q)+\frac{1}{4}\right\}M_0 - \frac{d}{2}\left\{(l+q)\left(l+q+\frac{1}{2}\right)\left(\log_{10}\overline{l+q}-\log_{10}\frac{1}{2}\right) - \frac{1}{3}\left(\overline{l+q}^3+\frac{9}{2}\overline{l+q}^2-\frac{9}{4}\overline{l+q}-\frac{1}{8}\right)\log_{10}e\right\}\right] \quad \text{(xii)}$$

$$b=\frac{6}{\left(l+q-\frac{1}{2}\right)^3}\left[2M_1-\left(l+q+\frac{1}{2}\right)M_0-\frac{c}{6}\left(l+q+\frac{1}{2}\right)\left(l+q-\frac{1}{2}\right)^3 - \frac{d}{2}\left\{(l+q)\left(\log_{10}\frac{1}{2}-\log_{10}\overline{l+q}\right)+\left(\overline{l+q}^2-\frac{1}{4}\right)\log_{10}e\right\}\right] \quad \text{(xiii)}$$

$$a=\frac{1}{l+q-\frac{1}{2}}\left[M_0-\frac{b}{2}\left\{\left(l+q\right)^2-\frac{1}{4}\right\}-\frac{c}{3}\left\{\left(l+q\right)^3-\frac{1}{8}\right\} - d\left\{(l+q)\log_{10}(l+q)-\frac{1}{2}\log_{10}\frac{1}{2}-\left(l+q-\frac{1}{2}\right)\log_{10}e\right\}\right] \quad \text{(xiv)}$$

For the curve

$$y=a+bx+c\log_{10}(x+\alpha)$$

the equations for determining the constants are:

$$c=\frac{6\left[\left(l+q+\frac{1}{2}\right)M_1-M_2\right]-\left[(l+q)^2+2(l+q)+\frac{1}{4}\right]M_0}{\left\{\left(\alpha+\frac{1}{2}\right)(l+q+\alpha)\left(l+q+2\alpha+\frac{1}{2}\right)\left[\log_{10}\left(\alpha+\frac{1}{2}\right)-\log_{10}(l+q+\alpha)\right] + \left(l+q-\frac{1}{2}\right)\left[\frac{1}{6}\left\{(l+q)^2+5(l+q)+\frac{1}{4}\right\}+2\alpha\left(l+q+\frac{1}{2}\right)+2\alpha^2\right]\log_{10}e\right\}} \quad \text{(xv)}$$

$$b=\frac{6}{\left(l+q-\frac{1}{2}\right)^3}\left[2M_1-\left(l+q+\frac{1}{2}\right)M_0-c\left\{\left(\alpha+\frac{1}{2}\right)(l+q+\alpha)\left(\log_{10}\alpha+\frac{1}{2}\right.\right. - \left.\left.\log_{10}\overline{l+q+\alpha}\right)+\frac{1}{2}\left(l+q-\frac{1}{2}\right)\left(l+q+2\alpha+\frac{1}{2}\right)\log_{10}e\right\}\right] \quad \text{(xvi)}$$

$$a=\frac{1}{l+q-\frac{1}{2}}\left[M_0-\frac{b}{2}\left\{(l+q)^2-\frac{1}{4}\right\}-c\left\{(l+q+\alpha)\log_{10}(l+q+\alpha) - \left(\alpha+\frac{1}{2}\right)\log_{10}\left(\alpha+\frac{1}{2}\right)-\left(l+q-\frac{1}{2}\right)\log_{10}e\right\}\right] \quad \text{(xvii)}$$

By the use of the third moment an equation might also be derived for determining α. As this, however, is a somewhat complex logarithmic expression, from which α can be obtained only after much labor, it has seemed best to determine α empirically, as in working by the method of least squares.

ORDINATES EQUALLY DISTRIBUTED

Up to this point we have considered that the ordinates were distributed at any irregular points on the base line. In the usual case, however, they will, of course, be at equal intervals from one another. When the ordinates are given at equal intervals, i. e., when $x_2-x_1=x_3-x_2=\cdots=x_m-x_{m-1}$, l becomes equal to the number of given ordinates, $q=\frac{1}{2}$, and the equations for the constants can be put in a somewhat simpler form.

For the curve $y=a+bx+c\log_{10}x$:

$$c=j_1[6\{(l+1)M_1-M_2\}-(l^2+3l+1.5)M_0] \qquad \text{(xviii)}$$

$$b=\frac{6}{l^3}[2M_1-(l+1)M_0-j_2c \qquad \text{(xix)}$$

$$a=\frac{1}{l}M_0-\frac{l+1}{2}b-j_3c \qquad \text{(xx)}$$

where

$$j_1=2\left[(l+1)\left(l+\frac{1}{2}\right)\left\{\log_{10}\frac{1}{2}-\log_{10}\left(l+\frac{1}{2}\right)\right\}+\frac{l}{3}(l^2+6l+3)\log_{10}e\right]^{-1}$$

$$j_2=\frac{3}{l^3}\left[\left(l+\frac{1}{2}\right)\left\{\log_{10}\frac{1}{2}-\log_{10}\left(l+\frac{1}{2}\right)\right\}+l(l+1)\log_{10}e\right]$$

$$j_3=\frac{1}{l}\left[\left(l+\frac{1}{2}\right)\log_{10}\left(l+\frac{1}{2}\right)-\frac{1}{2}\log_{10}\frac{1}{2}-l\log_{10}e\right]$$

For the curve $y=a+bx+cx^2+d\log_{10}x$:

$$d=j_4[20M_3-30(l+1)M_2+j_5M_1-j_6M_0] \qquad \text{(xxi)}$$

$$c=\frac{30}{l^5}[6\{M_2-(l+1)M_1\}+(l^2+3l+1.5)M_0]+j_7d \qquad \text{(xxii)}$$

$$b=\frac{6}{l^3}[2M_1-(l+1)M_0]-(l+1)c-j_2d \qquad \text{(xxiii)}$$

$$a=\frac{1}{l}M_0-\frac{l+1}{2}b-j_8c-j_3d \qquad \text{(xxiv)}$$

where

$$j_4=2\left[\left(l+\frac{1}{2}\right)\left(l^2+\frac{5}{2}l+\frac{5}{4}\right)\left\{\log_{10}\frac{1}{2}-\log_{10}\left(l+\frac{1}{2}\right)\right\}+\frac{l}{6}(l+1)\left(l^2+15l+\frac{15}{2}\right)\log_{10}e\right]^{-1}$$

$$j_5=12\left(l^2+\frac{5}{2}l+\frac{5}{4}\right)$$

$$j_6=(l+1)\left(l^2+5l+\frac{5}{2}\right)$$

$$j_7=\frac{30}{l^5}\left[\frac{1}{2}(l+1)\left(l+\frac{1}{2}\right)\left\{\log_{10}\frac{1}{2}-\log_{10}\left(l+\frac{1}{2}\right)\right\}+\frac{l}{6}(l^2+6l+3)\log_{10}e\right]$$

$$j_8=\frac{1}{3}\left(l^2+\frac{3}{2}l+\frac{3}{4}\right)$$

From the foregoing it is evident that since the j's involve only l, and with equal intervals for the ordinates l will always be some integer, the values of the j's for a series of values of l can be tabled once for all, and in this way a great deal of labor saved in the ordinary fitting of logarithmic curves. Accordingly tables of the j's and of $l^2+3l+1.5$, $\frac{30}{l^5}$, and $\frac{6}{l^3}$ have been prepared and are given in an appendix at the end of the paper.

For the curve $y=a+bx+c\log_{10}(x+\alpha)$:

$$c=\frac{6[M_2-(l+1)M_1]+(l^2+3l+1.5)M_0}{\alpha'(l+2\alpha')(l+\alpha')\{\log_{10}(l+\alpha')-\log_{10}\alpha'\}-\frac{l}{6}\{l^2+12\alpha' l+12\alpha'^2\}\log_{10}e} \quad \text{(xxv)}$$

$$b=\frac{6}{l^3}\Big[2M_1-(l+1)M_0-c\{\alpha'(l+\alpha')(\log_{10}\alpha'-\log_{10}l+\alpha')+\Big(\frac{l^2}{2}+\alpha' l\Big)\log_{10}e\}\Big] \quad \text{(xxvi)}$$

$$a=\frac{1}{l}M_0-\frac{l+1}{2}b-c\cdot\frac{1}{l}[(l+\alpha')\log_{10}(l+\alpha')-\alpha'\log_{10}\alpha'-l\log_{10}e] \quad \text{(xxvii)}$$

where $\alpha'=\alpha+\frac{1}{2}$.

ILLUSTRATIONS OF THE USE OF LOGARITHMIC EQUATIONS

In order to make clear the use of the above equations, some numerical illustrations will be given.

Let us first consider the data contained in Table I. These give the mean milk production in pounds over a 7-day period of Holstein-Friesian cattle at different ages. The data are taken from the official 7-day A. R. O. record of the Holstein-Friesian Association (11). The laborious task of extracting and tabulating these records and calculating the means was carried through by Mr. John W. Gowen, with the assistance of Mr. S. W. Patterson and Miss Anna B. Perkins, all of the Maine Experiment Station. In future publications from this laboratory these figures will be further dealt with, but here they are used solely for purposes of illustrating the method.

TABLE I.—*Mean 7-day milk production of Holstein-Friesian cows at different ages*

Age.	Number of cows.	Mean production.	Age.	Number of cows.	Mean production.
		Pounds.			*Pounds.*
1 yr. 6 mo. to 1 yr. 11 mo.	1,095	290.6	8 yr. to 8 yr. 5 mo.	434	467.0
2 yr. to 2 yr. 5 mo.	3,693	316.7	8 yr. 6 mo. to 8 yr. 11 mo.	432	466.6
2 yr. 6 mo. to 2 yr. 11 mo.	2,330	347.0	9 yr. to 9 yr. 5 mo.	249	464.6
3 yr. to 3 yr. 5 mo.	2,041	376.0	9 yr. 6 mo. to 9 yr. 11 mo.	243	462.5
3 yr. 6 mo. to 3 yr. 11 mo.	1,950	407.9	10 yr. to 10 yr. 5 mo.	149	460.7
4 yr. to 4 yr. 5 mo.	1,627	428.2	10 yr. 6 mo. to 10 yr. 11 mo.	137	460.2
4 yr. 6 mo. to 4 yr. 11 mo.	1,565	447.1	11 yr. to 11 yr. 5 mo.	72	455.2
5 yr. to 5 yr. 5 mo.	1,195	457.2	11 yr. 6 mo. to 11 yr. 11 mo.	67	453.7
5 yr. 6 mo. to 5 yr. 11 mo.	1,142	464.2	12 yr. to 12 yr. 5 mo.	37	449.1
6 yr. to 6 yr. 5 mo.	882	466.3	12 yr. 6 mo. to 12 yr. 11 mo.	35	444.0
6 yr. 6 mo. to 6 yr. 11 mo.	850	468.0	13 yr. to 13 yr. 5 mo.	20	443.2
7 yr. to 7 yr. 5 mo.	685	466.6	13 yr. 6 mo. to 13 yr. 11 mo.	22	448.7
7 yr. 6 mo. to 7 yr. 11 mo.	597	466.6	14 yr. to 14 yr. 5 mo.	10	440.0

The problem now is to fit by the method of moments a logarithmic curve of the form

$$y=a+bx+cx^2+d\log x$$

to these milk production means.

The calculations to obtain the moments are given in Table II.

TABLE II.—*Calculation of moments for data on Holstein-Friesian cows in original form*

Age.	y	y'	x'	$y'x'$	$y'x'^2$	$y'x'^3$
1 yr. 6 mo. to 1 yr. 11 mo..	290.6	326.06732	1	326.06732	326.06732	326.06732
2 yr. to 2 yr. 5 mo.....	316.7	240.32913	2	480.65826	961.31652	1,922.63304
2 yr. 6 mo. to 2 yr. 11 mo..	347.0	401.76094	3	1,205.28282	3,615.84846	10,847.54538
3 yr. to 3 yr. 5 mo.....	376.0	361.44305	4	1,445.77220	5,783.08880	23,132.35520
3 yr. 6 mo. to 3 yr. 11 mo..	407.9	407.9	5	2,039.5	10,197.5	50,987.5
4 yr. to 4 yr. 5 mo.....	428.2	428.2	6	2,569.2	15,415.2	92,491.2
4 yr. 6 mo. to 4 yr. 11 mo..	447.1	447.1	7	3,129.7	21,907.9	153,355.3
5 yr. to 5 yr. 5 mo.....	457.2	457.2	8	3,657.6	29,260.8	234,086.4
5 yr. 6 mo. to 5 yr. 11 mo..	464.2	464.2	9	4,177.8	37,600.2	338,401.8
6 yr. to 6 yr. 5 mo.....	466.3	466.3	10	4,663.0	46,630.0	466,300.0
6 yr. 6 mo. to 6 yr. 11 mo.	468.0	468.0	11	5,148.0	56,628.0	622,908.0
7 yr. to 7 yr. 5 mo.....	466.6	466.6	12	5,599.2	67,190.4	806,284.8
7 yr. 6 mo. to 7 yr. 11 mo.	466.6	466.6	13	6,065.8	78,855.4	1,025,120.2
8 yr. to 8 yr. 5 mo.....	467.0	467.0	14	6,538.0	91,532.0	1,281,448.0
8 yr. 6 mo. to 8 yr. 11 mo..	466.6	466.6	15	6,999.0	104,985.0	1,574,775.0
9 yr. to 9 yr. 5 mo.....	464.6	464.6	16	7,433.6	118,937.6	1,903,001.6
9 yr. 6 mo. to 9 yr. 11 mo..	462.5	462.5	17	7,862.5	133,662.5	2,272,262.5
10 yr. to 10 yr. 5 mo.....	460.7	460.7	18	8,292.6	149,266.8	2,686,802.4
10 yr. 6 mo. to 10 yr. 11 mo	460.2	460.2	19	8,743.8	166,132.2	3,156,511.8
11 yr. to 11 yr. 5 mo.....	455.2	455.2	20	9,104.0	182,080.0	3,641,600.0
11 yr. 6 mo. to 11 yr. 11 mo	453.7	453.7	21	9,527.7	200,081.7	4,201,715.7
12 yr. to 12 yr. 5 mo.....	449.1	449.1	22	9,880.2	217,364.4	4,782,016.8
12 yr. 6 mo. to 12 yr. 11 mo	444.0	426.81041	23	9,816.63943	225,782.70689	5,193,002.25847
13 yr. to 13 yr. 5 mo.....	443.2	513.14250	24	12,315.42000	295,570.08000	7,093,681.92000
13 yr. 6 mo. to 13 yr. 11 mo	448.7	340.49788	25	8,512.44700	212,811.17500	5,320,279.37500
14 yr. to 14 yr. 5 mo.....	440.0	493.70138	26	12,836.23588	333,742.13288	8,677,295.45488
Total.....	.....	11,315.45261	....	158,369.72291	2,806,320.01587	55,610,556.60929

For reasons which need not be considered here it is usually desirable to use corrected rather than raw values of the moments. Here we have used one of Elderton's (7) correction methods. The column headed y' is obtained from the y column by Elderton's formula V[2]—i. e., by multiplying the first and last ordinates by 1.1220486, the second and last but one by 0.7588542, the third and last but two by 1.1578125, and the fourth and last but three by 0.9612847.

From this table we have at once $M_0 = 11{,}315.45261$; $M_1 = 158{,}369.72291$; $M_2 = 2{,}806{,}320.01587$; $M_3 = 55{,}610{,}556.60929$; $l = 26$.

Substituting these values in the equations xxi to xxiv for the curve $y = a + bx + cx^2 + d \log x$, the following values for the constants are found:

$d = 0.000096940\ (20M_3 - 810M_2 + 8907M_1 - 21829.5M_0) = 259.83317.$

$c = 0.000025250\ (6M_2 - 162M_1 + 755.5M_0) + 0.0024102d = -0.0533.$

$b = 0.00034137\ (2M_1 - 27M_0) - 27c - 0.044239d = -6.225.$

$a = 0.0384615M_0 - 13.5b - 238.583333c - 1.022111d = 266.38.$

The final equation then becomes

$$y = 266.38 - 6.225x - 0.0533x^2 + 259.833 \log_{10} x.$$

The ordinates calculated from this equation in comparison with the observations are given in Table IV.

Before proceeding to any discussion of the fit, let us consider a second example, where the ordinates are at irregular intervals. The data here taken for illustration are the same as those of the preceding example, except that certain of the observations have been arbitrarily combined and the new values so obtained taken as ordinates. Table III shows

[2] It is to be noted that the coefficients as given by Elderton (7, p. 27) are incorrect in the last figure.

how this combination has been carried out, and also the calculation of the moments from ordinates at irregular intervals. Since in this instance the four ordinates at each end are regularly spaced, Elderton's formula V may be used. When the end ordinates are not regularly spaced, this is not applicable, and the ordinates may without serious loss of accuracy be left unmodified.

TABLE III.—*Calculation of moments for data on Holstein-Friesian cows, with grouping of certain ordinates*

Age.	y	y'	$h_{x'_k}$	$h_{x'_k}y'$	x'	$h_{x'_k}y'x'$	$h_{x'_k}y'x'^2$	$h_{x'_k}y'x'^3$
1 yr. 6 mo. to 1 yr. 11 mo...	290. 6	326. 07	1	326. 07	1	326. 07	326. 07	326. 07
2 yr. to 2 yr. 5 mo.....	316. 7	240. 33	1	240. 33	2	480. 66	961. 32	1,922. 63
2 yr. 6 mo. to 2 yr. 11 mo...	347. 0	401. 76	1	401. 76	3	1,205. 28	3,615. 85	10,847. 55
3 yr. to 3 yr. 5 mo.....	376. 0	361. 44	1	361. 44	4	1,445. 77	5,783. 09	23,132. 35
3 yr. 6 mo. to 3 yr. 11 mo...	407. 9	407. 9	1	407. 9	5	2,039. 5	10,197. 5	50,987. 5
4 yr. to 4 yr. 5 mo.....	428. 2	428. 2	1	428. 2	6	2,569. 2	15,415. 2	92,491. 2
4 yr. 6 mo. to 5 yr. 5 mo....	452. 1	452. 1	2	904. 3	7. 5	6,782. 25	50,866. 88	381,501. 56
5 yr. 6 mo. to 6 yr. 11 mo...	466. 2	466. 2	3	1,398. 5	10	13,985. 0	139,850. 0	1,398,500. 0
7 yr. to 7 yr. 11 mo.....	466. 6	466. 6	2	933. 2	12. 5	11,665. 0	145,812. 5	1,822,656. 25
8 yr. to 9 yr. 5 mo.....	466. 1	466. 1	3	1,398. 2	15	20,973. 0	314,595. 0	4,718,925. 0
9 yr. 6 mo. to 9 yr. 11 mo...	462. 5	462. 5	1	462. 5	17	7,862. 5	133,662. 5	2,272,262. 5
10 yr. to 10 yr. 5 mo.....	460. 7	460. 7	1	460. 7	18	8,292. 6	149,266. 8	2,686,802. 4
10 yr. 6 mo. to 11 yr. 11 mo.	456. 4	456. 4	3	1,369. 1	20	27,382. 0	547,640. 0	10,952,800.0
12 yr. to 12 yr. 5 mo.....	449. 1	449. 1	1	449. 1	22	9,880. 2	217,364. 4	4,782,016. 8
12 yr. 6 mo. to 12 yr. 11 mo.	444. 0	426. 81	1	426. 81	23	9,816. 64	225,782. 70	5,193,002. 26
13 yr. to 13 yr. 5 mo.....	443. 2	513. 14	1	513. 14	24	12,315. 42	295,570. 08	7,093,681. 92
13 yr. 6 mo. to 13 yr. 11 mo.	448. 7	340. 50	1	340. 50	25	8,512. 45	212,811. 17	5,320,279. 37
14 yr. to 14 yr. 5 mo.....	440. 0	493. 70	1	493. 70	26	12,836. 23	333,742. 13	8,677,295. 45
Total.....	.....	.....	.....	11,315. 45	.....	158,369. 77	2,803,263.19	55,479,430.81

From Table III we have the following values: $M_0 = 11{,}315.45$; $M_1 = 158{,}369.77$; $M_2 = 2{,}803{,}263.19$; $M_3 = 55{,}479{,}430.81$; $l+q = 26.5$; $l = 26$; $q = 0.5$.

Substituting these values in the equations xi to xiv, we obtain:

$d = 0.000096940\ (20M_3 - 810M_2 + 8907M_1 - 21{,}829.5M_0) = 245.67899.$

$c = 0.000025250\ (6M_2 - 162M_1 + 755.5M_0) + 0.0024102d = -0.1338.$

$b = 0.00034137\ (2M_1 - 27M_0) - 27c - 0.044239d = -3.425.$

$a = 0.0384615M_0 - 13.5b - 238.583333c - 1.022111d = 262.26.$

The final equation then becomes—

$$y = 262.26 - 3.425x - 0.1338x^2 + 245.679 \log_{10} x.$$

Table IV compares the fit of the two curves calculated by the method of moments with that of the curve fitted by the method of least squares. It is apparent that the two methods give results of substantially the same accuracy. By the method of moments the root mean-square error is greater, and the mean error less than by that of least squares. Neither difference is, however, large.

TABLE IV.—*Comparison of the graduation of data on Holstein-Friesian cows by different methods*

x	By least squares.				By moments.					
					Ordinates at equal intervals.			Ordinates at unequal intervals.		
	y from data.	y from curve.	Δ	Δ^2	y from curve.	Δ	Δ^2	y from curve.	Δ	Δ^2
1	290.6	272.1	+18.5	342.25	260.1	+30.5	930.25	258.7	+31.9	1,017.61
2	316.7	332.2	−15.5	240.25	331.9	−15.2	231.04	328.8	−12.1	146.41
3	347.0	366.9	−19.9	396.01	371.2	−24.2	585.64	368.0	−21.0	441.00
4	376.0	391.1	−15.1	228.01	397.1	−21.1	445.21	394.3	−18.3	334.89
5	407.9	409.2	− 1.3	1.69	415.5	− 7.6	57.76	413.5	− 5.6	31.36
6	428.2	423.4	+ 4.8	23.04	429.3	− 1.1	1.21	428.1	+ .1	.01
7	447.1	434.7	+12.4	153.76	439.8	+ 7.3	53.29	439.3	+ 7.8	60.84
8	457.2	443.8	+13.4	179.56	447.8	+ 9.4	88.36	448.2	+ 9.0	81.00
9	464.2	451.2	+13.0	169.00	454.0	+10.2	104.04	455.0	+ 9.2	84.64
10	466.3	457.7	+ 8.6	73.96	458.6	+ 7.7	59.29	460.3	+ 6.0	36.00
11	468.0	461.6	+ 6.4	40.96	462.0	+ 6.0	36.00	464.2	+ 3.8	14.44
12	466.6	465.1	+ 1.5	2.25	464.4	+ 2.2	4.84	467.0	− .4	.16
13	466.6	467.5	− .9	.81	465.8	+ .8	.64	468.8	− 2.2	4.84
14	467.0	469.0	− 2.0	4.00	466.6	+ .4	.16	469.7	− 2.7	7.29
15	466.6	469.6	− 3.0	9.00	466.6	0	0	469.7	− 3.1	9.61
16	464.6	469.4	− 4.8	23.04	466.0	− 1.4	1.96	469.0	− 4.4	19.36
17	462.5	468.5	− 6.0	36.00	464.9	− 2.4	5.76	467.7	− 5.2	27.04
18	460.7	466.9	− 6.2	38.44	463.2	− 2.5	6.25	465.7	− 5.0	25.00
19	460.2	464.5	− 4.3	18.49	461.1	− .9	.81	463.0	− 2.8	7.84
20	455.2	461.5	− 6.3	39.69	458.6	− 3.4	11.56	459.9	− 4.7	22.09
21	453.7	457.9	− 4.2	17.64	455.7	− 2.0	4.00	456.2	− 2.5	6.25
22	449.1	453.7	− 4.6	21.16	452.4	− 3.3	10.89	452.0	− 2.9	8.41
23	444.0	448.9	− 4.9	24.01	448.8	− 4.8	23.04	447.3	− 3.3	10.89
24	443.2	443.5	− .3	.09	444.9	− 1.7	2.89	442.1	+ 1.1	1.21
25	448.7	437.5	+11.2	125.44	440.7	+ 8.0	64.00	436.4	+12.3	151.29
26	440.0	431.0	+ 9.0	81.00	436.2	+ 3.8	14.44	430.4	+ 9.6	92.16
Total	.....	.....	198.1	2,289.55	.....	177.9	2,743.33	.....	187.0	2,641.64

Least squares: Root mean-square error=9.4; mean error=7.6.
Moments, equal intervals: Root mean-square error=10.3; mean error=6.8.
Moments, unequal intervals: Root mean-square error=10.1; mean error=7.2.

In Table V are given the values of the j's and certain other constants involving only l for values of l from 3 to 40. This range will include practically all cases likely to occur in ordinary statistical work.

The author wishes to acknowledge his indebtedness to Dr. Pearl for his aid and suggestions throughout the work.

TABLE V.—*Values of certain constants involving only l for values from 3 to 40*

l	j_1	j_2	j_3	$l^2+3l+1.5$	$\frac{6}{l^3}$	j_4	j_5	j_6	j_7	j_8	$\frac{30}{l^5}$
3	1.6701993	0.250410	0.250623	19.5	0.2222222						
4	.5832445	.205866	.338198	29.5	.0937500	0.5175122	327	192.5	0.0502309	7.583333	0.0292969
5	.2626105	.175228	.410207	41.5	.0480000	.1741179	465	315.0	.0365560	11.083333	.0096000
6	.1383150	.152774	.471447	55.5	.0277778	.0726607	627	479.5	.0278930	15.250000	.0038580
7	.0810052	.135567	.524773	71.5	.0174927	.0350722	813	692.0	.0220353	20.083333	.0017850
8	.0512108	.121936	.572028	89.5	.0117188	.0187981	1,023	958.5	.0178776	25.583333	.00091553
9	.0342957	.110857	.614471	109.5	.0082305	.0109020	1,257	1,285.0	.0148139	31.750000	.00050805
10	.0240239	.101667	.653006	131.5	.0060000	.0067235	1,515	1,677.5	.0124876	38.583333	.00030000
11	.0174459	.093915	.688300	155.5	.0045079	.0043556	1,797	2,142.0	.0106774	46.083333	.00018628
12	.0130483	.087284	.720863	181.5	.0034722	.0029376	2,103	2,684.5	.0092398	54.250000	.00012056
13	.0100021	.081545	.751092	209.5	.0027310	.0020488	2,433	3,311.0	.0080782	63.083333	.000080799
14	.0078282	.076527	.779302	239.5	.0021866	.0014700	2,787	4,027.5	.0071255	72.583333	.000055780
15	.0062369	.072102	.805749	271.5	.0017778	.0010807	3,165	4,840.0	.0063342	82.750000	.000039506
16	.0050464	.068168	.830643	305.5	.0014648	.00081130	3,567	5,754.5	.0056694	93.583333	.000028610
17	.0041386	.064649	.854157	341.5	.0012212	.00062037	3,993	6,777.0	.0051053	105.083333	.000021129
18	.0034347	.061480	.876438	379.5	.0010288	.00048211	4,443	7,913.5	.0046224	117.250000	.000015877
19	.0028809	.058612	.897610	419.5	.00087476	.00038009	4,917	9,170.0	.0042056	130.083333	.000012116
20	.0024392	.056003	.917779	461.5	.00075000	.00030353	5,415	10,552.5	.0038435	143.583333	.0000093750
21	.0020829	.053620	.937036	505.5	.00064788	.00024520	5,937	12,067.0	.0035266	157.750000	.0000073456
22	.0017923	.051434	.955461	551.5	.00056349	.00020016	6,483	13,719.5	.0032478	172.583333	.0000058211
23	.0015531	.049421	.973123	599.5	.00049314	.00016495	7,053	15,516.0	.0030012	188.083333	.0000046610
24	.0013544	.047562	.990084	649.5	.00043403	.00013711	7,647	17,462.5	.0027818	204.250000	.0000037676
25	.0011880	.045840	1.006397	701.5	.00038400	.00011487	8,265	19,565.0	.0025859	221.083333	.0000030720
26	.0010476	.044239	1.022111	755.5	.00034137	.000096940	8,907	21,829.5	.0024102	238.583333	.0000025250
27	.00092840	.042748	1.037267	811.5	.00030483	.000082359	9,573	24,262.0	.0022520	256.750000	.0000020908
28	.00082652	.041355	1.051905	869.5	.00027332	.000070406	10,263	26,868.5	.0021090	275.583333	.0000017431
29	.00073895	.040050	1.066059	929.5	.00024601	.000060535	10,977	29,655.0	.0019793	295.083333	.0000014626
30	.00066327	.038827	1.079761	991.5	.00022222	.000052326	11,715	32,627.5	.0018613	315.250000	.0000012346
31	.00059753	.037677	1.093038	1,055.5	.00020140	.000045457	12,477	35,792.0	.0017537	336.083333	.0000010479
32	.00054016	.036593	1.105916	1,121.5	.00018311	.000039673	13,263	39,154.5	.0016552	357.583333	.00000089407
33	.00048987	.035571	1.118418	1,189.5	.00016696	.000034777	14,073	42,721.0	.0015649	379.750000	.00000076657
34	.00044560	.034605	1.130567	1,259.5	.00015266	.000030610	14,907	46,497.5	.0014818	402.583333	.00000066028
35	.00040650	.033690	1.142380	1,331.5	.00013994	.000027046	15,765	50,490.0	.0014052	426.083333	.00000057119
36	.00037181	.032823	1.153878	1,405.5	.00012860	.000023984	16,647	54,704.5	.0013344	450.250000	.00000049615
37	.00034095	.032000	1.165075	1,481.5	.00011845	.000021341	17,553	59,147.0	.0012689	475.083333	.00000043263
38	.00031340	.031218	1.175988	1,559.5	.00010935	.000019051	18,483	63,823.5	.0012081	500.583333	.00000037862
39	.00028872	.030473	1.186631	1,639.5	.00010115	.000017059	19,437	68,740.0	.0011517	526.750000	.00000033251
40	.00026656	.029763	1.197017	1,721.5	.00009375	.000015320	20,415	73,902.5	.0010991	553.583333	.00000029297

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ORGANIC PHOSPHORIC ACID OF RICE

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That phosphoric acid occurs in organic combinations with inosite in the seeds of many plants has been shown by several investigators. Posternak (9),[1] Patten and Hart (8), Hart and Andrews (6), Hart and Tottingham (7), Anderson (1, 2, 3, 5), Rather (10, 11), and others have isolated this organic substance from pumpkin seed, beans, wheat, corn, oats, and cotton seed. Although they have been unable to obtain it by synthesis and still disagree as to the formula and composition of the acid and its salts, it is generally known as phytin or phytic acid. Suzuki et al. (12, 13) and Anderson believe phytin to be a hexaphosphoric acid ester of inosite, and Anderson has shown that the organic phosphoric acid in wheat bran differs materially from the acid he has obtained from a number of seeds.

Phytin is completely hydrolyzed into free phosphoric acid and inosite only with difficulty, as Anderson (4) showed by boiling phytin with concentrated nitric acid for several hours.

In the previous determination of phosphoric acid in foliage and grain of rice (*Oryza sativa*) at the Hawaii Experiment Station (14) several methods were used in oxidizing the organic matter. On boiling the grain with a mixture of nitric and hydrochloric acids (aqua regia) the author noticed that, although the solution soon became colorless, giving the appearance of complete oxidation of organic matter, if boiled to dryness a charred mass remained in the flask. Determinations of phosphoric acid in the solution (not boiled to dryness) in the case of the rice grain showed about one-third of the total phosphoric acid as found by the Neumann method. The determination of phosphoric acid in the foliage, on the other hand, by either method was about the same.

It was thought that the reason for this resistance to the action of aqua regia is probably the fact that phosphoric acid occurs in the rice grain as phytin and is therefore not completely hydrolyzed. It was decided, therefore, to give some study to the organic phosphoric acid of rice.

Suzuki et al. (12, 13) obtained an impure salt of phytic acid from rice by extracting the rice bran with a 0.2 per cent hydrochloric-acid solution and precipitating with alcohol. As Anderson (1, 2, 3, 4, 5) has shown that phytin so prepared would contain the inorganic phosphoric acid of the seed, as well as other impurities, the phosphorus content as shown by the resulting analysis is not that of pure phytin.

It is therefore of interest to obtain the pure salt of phytic acid from rice. In following the methods of Anderson (1, 2, 3, 4, 5) of purifying the acid by repeated solution in hydrochloric acid and precipitation with barium hydroxid, the author hoped to isolate the pure tribarium salt, but the ease of partial hydrolysis of the substance and the difficulty of eliminating all impurities which may be present, such as other phos-

[1] Reference made by number to "Literature cited," p. 430.

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phoric esters of inosite, stand in the way of obtaining the pure substance. Special attention was paid to methods for the determination of the barium and phosphoric acid in the salt, and observations of some interest were made.

The total phosphorus was determined in samples of rice bran and unpolished and polished rice. The following determinations were duplicated to within 0.02 per cent: Phosphorus in rice bran, 2.291 per cent; in unpolished rice, 0.321 per cent; in polished rice, 0.140 per cent.

It is apparent that the rice bran contains a comparatively high percentage of phosphorus. Phytin was determined in the bran by extracting 100 gm. of the sample with a 0.2 per cent hydrochloric-acid solution and precipitating with alcohol. The precipitate stood over night, when it was filtered, first washed with 50 per cent alcohol, then with ether, and was dried at 105° C. It weighed 8.22 gm., amounting, therefore, to 8.22 per cent of the bran. As phytin contains considerable organic phosphorus, it is apparent that rice bran contains much of its phosphorus in the organic form.

The writer was unable to obtain phytin from the polished rice. The 0.2 per cent hydrochloric-acid extract from several kilograms of finely ground polished rice yielded but a slight precipitate with alcohol or barium chlorid. With barium hydroxid added to alkalinity a considerable precipitate occurred which did not behave like phytin.

The phytin obtained from the unpolished rice was doubtless contained in the outer layer, which is removed in polishing.

Two preparations of barium phytate were made according to the method of Anderson; one from unpolished rice, the other from rice bran.

Six kg. of finely ground unpolished rice were treated with a 0.2 per cent hydrochloric-acid solution for several hours. This was filtered through cheesecloth and filter paper the same day and the filtrate precipitated with a barium-chlorid solution.

The precipitate was collected on a filter, washed with 50 per cent alcohol, dissolved in a 1.0 per cent hydrochloric-acid solution, which was then filtered and precipitated with barium hydroxid. It was reprecipitated three additional times with barium hydroxid, once with alcohol, again with barium hydroxid, and again three times with alcohol. The filtrate from the alcohol precipitate soon ceased to give an immediate precipitate with an ammonium-molybdic solution, showing the elimination of inorganic phosphoric acid from the precipitate. The precipitates formed by barium hydroxid were well washed with water.

Crystals were then obtained by adding barium hydroxid to the acid solution until a slight precipitate resulted and then filtering and allowing to stand a couple of days. The crystals thus formed were washed, recrystallized in the same way, and then crystallized from the acid solution by the addition of alcohol. It was found that on adding an equal volume of alcohol, according to Anderson, only an amorphous precipitate was obtained. It was only by adding just sufficient alcohol to make the solution turbid that crystallization took place.

Under the microscope the precipitate appeared to be composed entirely of crystals in the form of globules of needles with occasional needle-shaped crystals arranged in stellar form. These were probably the same substance.

The crystals were washed with alcohol and ether, dried in vacuum over sulphuric acid at laboratory temperature, and finally at 105° to 110° C.

over calcium chlorid without vacuum. The moisture was then determined in the salt at 120° to 130° C. and amounted to 0.76 per cent. The phytin from the bran was purified and obtained in the same way as that from unpolished rice, but the barium salt was dried in vacuum over calcium chlorid at 105° C. The moisture determined in vacuum at 105° and at 120° C. was 1.33 per cent in both cases. The crystals resembled those of the first preparation.

The salts thus obtained were practically free from chlorids and inorganic phosphates. Nitrogen was also absent. All the material of the first preparation was used in making repeated determinations of barium, phosphorus, carbon, and hydrogen, but the phytin obtained from the bran was analyzed also for ash constituents other than barium. In 0.60 gm. of this material an unweighable trace of calcium was found, but no iron, manganese, magnesium, or potash. The residue on precipitating out the barium and igniting the phytic acid thus left amounted to a few milligrams and was composed mostly of unvolatilized phosphoric acid. No nitrogen was found in the salt.

The barium in the salts was determined by various methods. A precipitate of barium sulphate was obtained in the determination of phosphorus by the Neumann method. After boiling the salts for three to four hours with sulphuric acid to which ammonium nitrate was added at intervals, 200 c. c. of water were added to the solution, and the precipitate of barium sulphate resulting from this dilution was boiled and allowed to stand overnight on the water bath. The precipitate was collected on a filter and was washed well, dried, and ignited. This precipitate was not pure barium sulphate, but contained a large amount of silica, aluminum, etc., dissolved from the Kjeldahl flask by the concentrated solution of very hot ammonium nitrate in sulphuric acid. On heating the weighed precipitate with hydrofluoric and sulphuric acids, silica was eliminated and the weight of the residue considerably reduced. In one case 0.6853 gm. of precipitate lost 0.0111 gm. by this treatment. A pure barium sulphate was also obtained by fusing the residue with sodium carbonate. The fusion was digested in boiling water, then filtered and washed. The filtrate was tested for barium and the insoluble residue dissolved in a few drops of dilute hydrochloric acid, washed through the filter paper, and the barium precipitated in boiling solution by the slow addition of 0.4 *N* sulphuric acid.

A number of determinations were thus made in which the correction by fusion caused a decrease in calculated barium from 1 to 2 per cent. The results from three samples given in Table I are taken from the analysis of the phytin from bran (not calculated to moisture-free basis).

TABLE I.—*Percentage of barium in barium phytate oxidized by the Neumann method*

Sample No.	Barium.	
	Uncorrected.	Corrected by fusion.
	Per cent.	*Per cent.*
1	38.293	37.291
2	38.256	37.358
3	38.191	37.305

Two of the corrected residues were treated with hydrofluoric acid, but no decrease in weight resulted.

Barium was also determined by dissolving the barium phytate in about 200 c. c. of water with a few cubic centimeters of dilute hydrochloric acid. Twenty c.c. of 0.4 *N* sulphuric acid were added to the boiling solution, the whole was boiled about half an hour, and then set aside on a hot-water bath for several hours. The precipitate, after standing overnight, was filtered, washed, and ignited. On weighing, then fusing and reprecipitating the barium, as was done above, a slight increase in weight was observed. The filtrates from the barium precipitates after fusion were united, evaporated to small bulk, acidified with nitric acid, and ammonium molybdate added. A yellow precipitate was found after standing, and the phosphoric acid then obtained was weighed as magnesium pyrophosphate. By fusing the impure residues amounting to from 0.2754 to 0.3511 gm., the magnesium pyrophosphate obtained was found to be 0.0181, 0.0092, 0.0095, and 0.0074 gm.

The weight of the residues of barium sulphate before and after correction for phosphate is given in Table II.

TABLE II.—*Quantity of barium sulphate* [a] *and magnesium pyrophosphate precipitated from a solution of barium phytate*

Samples No.	Uncorrected.	Fused and corrected.	Phosphoric acid as magnesium pyrophosphate.
	Gm.	*Gm.*	*Gm.*
1	0.2754	0.2785	0.0181
2	.3117	.3128	.0092
3	.4672	.4703	.....

[a] Not calculated to water-free basis.

The filtrate containing the phytic acid from which the barium was precipitated by the foregoing method still contained a few tenths of a per cent of barium. On evaporating to small bulk or igniting, a small precipitate of barium sulphate was obtained. It would appear that phytic acid has some solvent action on barium sulphate.

The fact that phosphoric acid was precipitated along with the barium sulphate by very dilute sulphuric acid suggests that the composition of phytic acid as determined by Anderson may have been affected. The phosphorus, if carried down with the barium sulphate, would cause a low phosphorus content in the remaining solution.

It was attempted to determine barium by first igniting the salt, but a white ash could not be obtained, and the residue was extremely difficult to dissolve after ignition. Phosphoric acid was best determined by the Neumann method, filtering off the barium sulphate formed on dilution. The results were about 0.1 per cent lower when determined by precipitating the barium with dilute sulphuric acid and evaporating the phytic acid thus obtained with magnesium nitrate and igniting and adding the phosphorus anhydrid found in the fused barium residue.

Carbon and hydrogen were determined by the regular combustion method, passing oxygen through the apparatus during the burning. In each case the black ash remaining was ground up with potassium dichromate and reburned.

The analyses of the barium phytate are given in Table III and are calculated to the water-free basis. The barium and phosphorus content is lower than that reported by Anderson for tribarium-inosite-hexaphosphate. Whether the barium phytate obtained was composed of a single salt of inosite is not absolutely certain.

TABLE III.—*Analyses of barium phytate calculated to the water-free basis*

Constituent.	Source of barium phytate.			
	Unpolished rice.		Rice bran.	
	Sample No. 1.	Sample No. 2.	Sample No. 1.	Sample No. 2.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
C	6.63	6.97	6.62	6.51
H	1.75	1.84	1.82	1.87
P	16.43	16.38	16.06	16.05
Ba	36.93	36.84	37.79	37.84

UNPOLISHED RICE [a]

Sample No.	Quantity used.	H_2O	CO_2	$Mg_2P_2O_7$	$BaSO_4$
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	0.3729	0.0607	0.0900	.....	.....
2	.3551	.0605	.0901	.....	.....
1	.4752	.....	.....	0.2779	.....
2	.2365	.....	.....	.1379	.....
1	.4514	.....	.....	.....	0.2811
2	.5042	.....	.....	.....	.3132

RICE BRAN [b]

Sample No.	Quantity used.	H_2O	CO_2	$Mg_2P_2O_7$	$BaSO_4$
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	0.2559	0.0445	0.0613	.....	.....
2	.3271	.0583	.0771	.....	.....
1	.5514	.....	.....	0.3137	.....
2	.4765	.....	.....	.2709	.....
1	.5514	.....	.....	.....	0.3496
2	.6730	.....	.....	.....	.4251

[a] 0.5107 gm. lost 0.0039 gm. H_2O=0.76 per cent of moisture.
[b] 0.9717 gm. lost 0.0129 gm. H_2O=1.33 per cent of moisture.

Inosite was prepared from the barium phytate of rice bran by heating in sealed tubes to 150° C. about 2 gm. of the salt with 20 c. c. of 30 per cent sulphuric acid for five hours.

The sulphuric acid was precipitated with barium hydroxid, the excess of barium removed by carbon dioxid, and the filtrate evaporated to dryness. The residue was extracted with hot water and filtered. The inosite was precipitated by ether and alcohol and recrystallized three times as minute needles. These gave the Scherer reaction and melted at 223° C., uncorrected.

Thanks are due Dr. W. P. Kelley, of the Hawaii Experiment Station, who suggested this work on phytin in rice and gave helpful advice throughout.

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TWO CLOVER APHIDS[1]

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It recently came to my attention that two distinct species of clover aphids are rather generally confused in collections under the name "*Aphis bakeri*." As the range of both species extends nearly, if not quite, across the continent, it is a matter of more than local interest that this confusion should be straightened out. Specimens have been received from Messrs. J. J. Davis, A. Maxson, and H. F. Wilson, and I am indebted to Prof. C. P. Gillette for reading the manuscript and for the determination as to which species should properly be known as *Aphis bakeri*.

I have been unable to secure a type specimen of *Aphis brevis* for examination, although through the kindness of Dr. L. O. Howard, Chief of the Bureau of Entomology, and Mr. H. Hayward, Director of the Delaware Experiment Station, a thorough search was made both at Washington and at Newark. I feel confident, however, that Prof. Sanderson's careful description and figures[2] are sufficient to enable us to refer the long-beaked clover aphid to that species with safety.

Aphis brevis Sanderson (Long-beaked clover aphid).

In the vicinity of Orono, Me., the leaves of the hawthorn (*Crataegus* spp.) in June are commonly twisted into dark-purple swollen curls and are inhabited by an aphid the fall migrants of which were described by Prof. Sanderson as *Aphis brevis*.[2] This insect takes flight from hawthorn during June and early July and returns late in the season before producing the sexual generation. I have taken the fall migrants on cultivated plum (*Prunus* spp.), but as yet have made no spring collections from that host. In June and July, 1906, I collected apparently the same species from the twigs and terminal leaf curls of the Japan quince (*Cydonia japonica*).

Not being able to find characters to separate these collections from certain specimens labeled "*Aphis bakeri*" received from the Middle West, I undertook some transfer tests during the summer of 1912, and found that my *Aphis brevis* accepted both alsike and other clover (*Trifolium* spp.). Migrants placed on alsike and white clover produced nymphs that fed with apparent satisfaction on the test plants. The potted white clover was, however, more easily managed in the laboratory, so it was selected for the main rearings. The transfer was made on June 14. The migrants fed on the clover, and their abdomens became distended. At this time the head, thorax, and cornicles were black, and abdomens olive green, with distinct black lateral dots. By June 21 their abundant progeny were established on both stem and runner. The nymphs at first were pale and pellucid, with rosy head and prothorax. By June 24 this generation had matured, but did not begin to reproduce for a day or two. By June

[1] Papers from the Maine Agricultural Experiment Station: Entomology No. 76.
[2] Sanderson, E. Dwight. Report of the entomologist. *In* Del. Agr. Exp. Sta. 13th Ann. Rpt. [1900] /01, p. 157–158. 1902.

27 these adults had lost the rosy hue they had as nymphs and had become creamy white or grayish white with black antennæ, dusky legs, and deep rusty spots at base of cornicles, but with no rusty line connecting them.

On August 5, 1912, my attention was called to infested sweet-pea (*Lathyrus odoratus*) vines, which had vigorous colonies of red aphids on the stems at the surface of the ground extending for an inch up the plants. These proved to be the long-beaked clover aphids, and the source of the infestation doubtless was the hawthorn tree a few rods distant which had been heavily attacked by the spring generations of this species earlier in the season.

The spring forms on the hawthorn include two types of apterous females. One, possibly the stem mother, has the head, prothorax, and thorax soft coral pink. Joints I, II, and III of the antenna are coral pink or pellucid, while the other joints are black. The abdomen is olive green mottled with brown and pink, with a slight bloom only. The very short cornicle is light pellucid with the merest dusky tip, and the very short cauda is dark brown.

The apterous female of the second (or third?) generation has the head, prothorax, and thorax crimson, overcast with a slight bloom. Joints I, II, and III of the antenna are pellucid, while IV, V, and VI are black. The abdomen is crimson mottled with olive green and

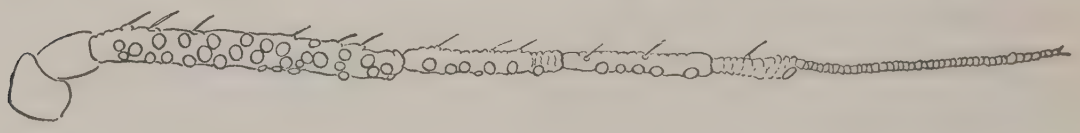

FIG. 1.—*Aphis brevis:* Antenna of fall alate female collected from hawthorn.

covered with slight bloom. There is a pale olive space about the base of the short cornicles, which are light olive green.

The spring migrant before flight has the dorsal surface of the head shining black, the ventral reddish, with a black beak and the antennæ black; the prothorax is black with red membrane; the dorsal lobes of the thorax are shining black, the breast reddish black; the dorsum of the abdomen is red in form of a heavy cross, the part about the cornicles being pale olive green; the venter is red. These same migrants after feeding on clover juice lose with age their reddish cast, the abdomens then becoming olive green.

The nymph, which is to become the spring migrant, in the pre-imago stage has its head and thorax coral pink, its abdomen red and more or less mottled, with a pale olive green space about the cornicle extending over several segments.

The fall alate form does not differ essentially from the spring migrant. The sensoria of the antenna (fig. 1) are distributed over joints III, IV, and V, in practically the same numbers as the spring migrant, although some individuals of the spring form have fewer to none on joint V. V and VI are nearly subequal, and they are both sometimes longer than those shown in figure 1. Joint V is sometimes a little shorter than IV.

Joints IV, V, and VI in the male are relatively longer than those in the alate female and there are frequently more sensoria on IV and V, as well as sometimes from one to several on basal part of joint VI (fig. 2).

The lateral tubercles of the prothorax and abdomen are distinct and blunt in both the alate and the apterous generations.

The beak in the apterous forms ordinarily reaches easily to the second coxa, and in the alate forms well beyond the second coxa, sometimes reaching the third. The most distinctive character of the wing is the short, broad stigma with a blunt distal end.

Aphis bakeri Cowen (Short-beaked clover aphid).

About the middle of August, 1914, large numbers of an aphid from *Trifolium pratense* were taken by Mr. George Newman at Orono, Maine. This species was distinct from the one just discussed, and yet I found that it was commonly listed in collections as *Aphis bakeri*. In the

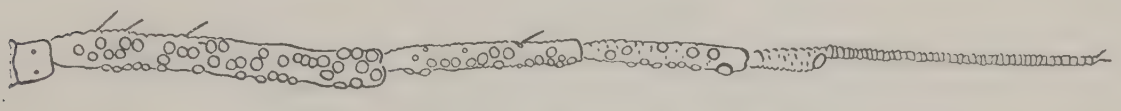

FIG. 2.—*Aphis brevis:* Antenna of alate male.

original description of *Aphis cephalicola* Cowen,[1] a synonym of *A. bakeri*, according to Gillette and Taylor,[2] the specifications "Third joint of antennæ tuberculate, with numerous irregular sensoria, fourth with few irregular sensoria," and "Beak hardly reaching second coxa" at once applied to *A. bakeri* of this paper and distinctly did not apply to *A. brevis*. *Aphis bakeri* is found also upon shepherd's-purse (*Capsella bursa-pastoris*) in the fall and early spring, but whether there is a migration between shepherd's-purse and clover I do not know. Mr. Wilson lent me specimens of this aphid collected from the hawthorn in Oregon. It occurs on apple (*Malus* spp.) in Colorado.[2] I have made a single collection of a fall migrant on hawthorn at Orono on October 1, 1914.

The habitat of the short-beaked clover aphid on clover seemed to be the ventral side of the leaf and the stem near the ground. The colonies

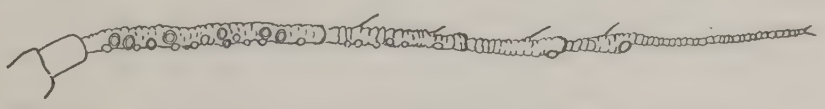

FIG. 3.—*Aphis bakeri:* Antenna of alate female collected from clover.

were frequently covered by "ant sheds," as well as sometimes extending for a short distance underground.

This species is smaller, more slender and graceful than the long-beaked clover aphid. Joint V of the antenna is noticeably shorter than IV and is without sensoria, except the usual distal one, in the summer winged viviparous female (fig. 3.) The stigma is rather narrow and the distal end acute. The beak hardly reaches the second coxa and frequently falls considerably short of it. The prothoracic and abdominal lateral tubercles are prominent, but very slender. Both species have the cornicles and cauda very short.

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NET ENERGY VALUES OF FEEDING STUFFS FOR CATTLE

By HENRY PRENTISS ARMSBY, *Director*, and J. AUGUST FRIES, *Assistant Director*, *Institute of Animal Nutrition of The Pennsylvania State College*

COOPERATIVE INVESTIGATIONS BETWEEN THE BUREAU OF ANIMAL INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE AND THE INSTITUTE OF ANIMAL NUTRITION OF THE PENNSYLVANIA STATE COLLEGE

INTRODUCTION

Besides supplying certain specific forms of matter (ash ingredients, proteins, lipoids, carbohydrates, vitamines, etc.) essential to the normal course of metabolism, the feed of an animal is, so far as we know, the sole source of the energy whose transformations constitute the essential phenomena of physical life. This energy is contained in the feed as chemical energy, and the maximum quantity which any substance can furnish for the vital activities by its oxidation in the body is measured by its heat of combustion. It rarely, if ever, happens, however, that this maximum effect is realized. In practically every case a larger or smaller proportion of the chemical energy of the feed escapes unutilized. These losses of energy are of two general classes.

First, a portion of the chemical energy of the feed fails to be transformed at all, leaving the body as chemical energy in the visible excreta and in the combustible gases arising from gastric and intestinal fermentations.

Second, another portion of the chemical energy of the feed is indeed transformed, but at ordinary temperatures virtually results merely in a superfluous heat production. It is true that the metabolism consequent upon feed consumption is not only unavoidable but may be regarded as a necessary expenditure of energy for the support of the activities connected with digestion and assimilation. Nevertheless, from the standpoint of the net gain or loss by the organism this portion of the feed energy, which ultimately takes the form of heat and escapes from the body, must be regarded as a loss.

The remainder of the chemical energy in the feed, after deducting these two classes of losses, has been designated as its net energy value and

expresses the net effect of the feed either in causing a storage of chemical energy in the form of fat, protein, etc., in the body, or, in the case of a submaintenance ration, in diminishing the amount of energy which must be supplied by the katabolism of body tissue.

The investigations of the last 30 years have shown that both the general problems of nutrition and the economic questions relating to the feeding of domestic animals may be advantageously studied from the standpoint of energetics. From this standpoint, it is of importance to determine as accurately as may be the losses of energy which feed substances undergo in the two ways just mentioned and the resulting net energy values. In the following pages are reported the results of a considerable number of experiments on cattle carried out at this Institute during the years 1902 to 1912, inclusive, in which these losses have been determined for certain feeding stuffs.[1] These experiments up to the end of 1907 have been already reported in full (7, 8, 9, 10) [2] and it is hoped to discuss the details of the later ones in subsequent papers. Here it will be convenient, following a general description of the experiments, to consider:

I. The losses of chemical energy.
II. The expenditure of energy consequent upon feed consumption and its factors.
III. Net energy values and their computation.

GENERAL DESCRIPTION OF THE EXPERIMENTS

The experiments were made with the aid of a respiration calorimeter of the Atwater-Rosa type, the essential features of which have already been described (3, 4, 7). The apparatus permits a determination of the water vapor and carbon dioxid excreted, of the carbon and hydrogen in the combustible gases produced, and of the heat given off, but not of the oxygen consumed. In addition to the ordinary feeding stuffs analyses of feed and excreta, the quantitative collection of the feces and urine and the determination of the amounts of carbon, hydrogen, and energy contained in them were also necessarily involved. The experiments comprised, in all, 76 single feeding periods. Each period covered at least 3 weeks, of which 11 days or more constituted a preliminary period, while the visible excreta were collected for the last 10 days, during which, on the seventh and eighth days, the complete balance of matter and energy was determined for 48 consecutive hours in the respiration calorimeter.[3] The accuracy of this instrument was tested by means of numerous alcohol checks. The results of 18 such checks (10, p. 217–222) showed that the

[1] In all, over 30 persons have taken a more or less direct part in the respiration trials and in the large amount of analytical, clerical, and miscellaneous work involved in the experiments. For obvious reasons, it is impossible even to attempt any statement of the exact part taken by individuals or to make acknowledgments for the specific work done by each person. This is all the more true because the most important factor in whatever success the investigation has attained, and one which by its nature is incapable of such partition, is the loyalty and zeal which all concerned have shown in the execution of the plan of the investigation and in securing the greatest attainable accuracy of details.

[2] Reference is made by number to "Literature cited," p. 489–491.

[3] For details regarding the methods employed compare the bulletins of the Bureau of Animal Industry already cited (7, 8, 9, 10), especially Bulletin 128 (10), p. 200–216, as well as the detailed descriptions of the single experiments contained in that bulletin.

values obtained in a single experiment may be regarded as accurate to within the following percentages of the amounts determined: Carbon dioxid, 0.5; water, 6.0; heat, 1.0.

A further test of the accuracy of the work is found in a comparison of the observed heat production with that computed in the ordinary way from the balance of carbon and nitrogen. Comparisons of this sort for 57 feeding periods up to the end of 1909 (5, 6) showed an average difference of 0.4 per cent. The results of the later comparisons reduce this difference to 0.3 per cent, or, if the unsatisfactory results of the year 1905 be omitted, to 0.04 per cent. The total amounts of heat involved are as follows:

	76 periods.	68 periods.
Computed heat production ..... Calories..	1,338,887	1,231,711
Observed heat production ..... do....	1,343,071	1,231,251
Difference ..... do....	4,184	460
Percentage difference.....	0.31	0.04

While the basis for the computation of the heat production is not altogether satisfactory, especially in the absence of determinations of the gain or loss of glycogen by the animal, nevertheless the general agreement is such as apparently to preclude the existence of gross errors.

FEEDING STUFFS

The dry matter of the several feeding stuffs used had the following average composition, as shown in each case by concordant analyses of two or more separate samples taken at the beginning of each experiment (Table I). The more important determinations were also repeated upon samples taken when the feed was weighed out for each period, and these period results form the basis of the computations on subsequent pages.

TABLE I.—*Composition of the dry matter of the feeding stuffs*

Feeding stuff and experiment No.	Ash.	Protein.	Non-protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.	Heat of combustion per kilogram.
Timothy hay:	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Calories.*
174.....	4.64	5.11	0.24	38.92	49.01	2.08	4,554
190.....	4.87	5.08	.39	38.15	49.51	2.01	4,431
200.....	5.86	6.72	.83	33.02	51.01	2.56	4,516
207.....	5.01	6.90	.24	31.15	54.55	2.15	4,505
Red clover hay:							
179.....	6.40	12.90	1.61	31.61	44.81	2.67	4,457
186.....	6.57	11.18	.89	28.78	49.65	2.93	4,492
Mixed hay:							
211.....	7.01	9.62	1.29	33.78	46.00	2.30	4,396
Alfalfa hay:							
208.....	9.40	11.86	2.52	31.96	42.75	1.51	4,403
209.....	10.76	12.09	1.67	31.70	41.86	1.92	4,330
212.....	9.06	12.39	2.86	30.10	43.63	1.96	4,368
Alfalfa meal:							
212.....	9.24	11.75	2.87	31.12	43.17	1.85	4,374
Maize stover:							
210, total.....	6.14	4.09	.86	36.15	51.46	1.30	4,337
210, portion eaten	6.67	4.46	.98	35.23	51.25	1.41	4,332

TABLE I.—*Composition of the dry matter of the feeding stuffs*—Continued

Feeding stuff and experiment No.	Ash.	Protein.	Non-protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.	Heat of combustion per kilogram.
Maize meal:	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Calories.*
179	1.37	9.94	.48	2.60	81.38	4.23	4,431
211	1.62	9.29	.24	3.16	80.64	5.05	4,517
Wheat bran:							
190	7.52	14.50	.49	11.49	61.88	4.12	4,532
Grain mixture No. 1:[a]							
200	4.10	16.97	3.43	6.09	62.89	6.52	4,690
207	4.14	17.95	2.22	6.16	64.38	5.15	4,670
Grain mixture No. 2:[b]							
208	2.78	12.07	.78	5.63	73.65	5.09	4,604
209	2.54	13.21	.54	5.81	72.77	5.13	4,617
Hominy chop:							
211	2.75	9.33	1.29	5.13	72.65	8.85	4,709

[a] Wheat bran, 14.28 per cent; maize meal, 42.86 per cent; old process linseed meal, 42.86 per cent.
[b] Maize meal, 60 per cent; crushed oats, 30 per cent; old process linseed meal, 10 per cent.

ANIMALS

Nine different steers have been used, varying in age from 11 months to approximately 60 months at the beginning of the several experiments.[1] They were either full bloods or high grades of recognized beef breeds, with one exception, steer B, which was distinctly of the dairy type and of mixed breeding (scrub), Jersey blood apparently predominating. All were docile animals and were thoroughly accustomed to the necessary handling, to wearing the apparatus for the collection of excreta, and to their surroundings in the digestion stall and the calorimeter. Further particulars concerning them are contained in Table II.

TABLE II.—*Description of the animals used in the experiments*

Animal No.	Breed of animal.	Nos. of experiments in which used.	Age at beginning of each experiment.	Average live weight in each experiment.
			Months.	*Kilograms.*
I	Grade Shorthorn	174	36	408
		179	48	528
		186	60	572
A	Aberdeen Angus	190	11	274
		200	23	408
		207	35	510
B	Scrub	190	13	195
		200	25	303
		207	37	380
C	Grade Hereford	208	9	275
D	do	208	9	167
		210	21	331
		211	33	449
E	do	208	9	208
F	do	209	21	300
G	Full-blood Hereford	211	28	379
H	Full-blood Shorthorn	212	20	345

[1] The word "experiment" is here used to designate the work of an entire season, including several feeding periods.

I. LOSSES OF CHEMICAL ENERGY—METABOLIZABLE ENERGY

The losses of chemical energy occur substantially in the feces and urine and in the combustible gases.

The energy content of the dried feces and urine is readily determined. In investigations at this Institute, Braman (16) has shown that the loss of energy in the drying of urine may be estimated with a good degree of accuracy, the error being insignificant in comparison with the total energy of the feed. The possible loss of energy in the drying of the feces has not yet been investigated directly, although Fingerling, Köhler, and Reinhardt (18) have observed a loss of carbon, the amount of which they do not state.

The energy content of the combustible gases is not susceptible of direct determination, but must be estimated from their chemical composition. The combustible gases which have been actually identified as excreted by cattle are methane and hydrogen. All investigations are in accord in showing that the former is the chief product of the normal fermentations occurring in the digestive tract, but results differ regarding the extent to which hydrogen is formed. In our experiments the gases were analyzed by passing them over platinized kaolin at a red heat. By this method in almost every instance a ratio of C to H slightly greater than that in CH_4 (2.976 to 1) has been found, the average of 57 experiments reported by the junior author elsewhere (19) being 3.167 to 1, with considerable variations in individual cases. We are inclined to think that this high figure is due to failure to oxidize the last traces of hydrogen in the combustion tube. On the other hand, Markoff (37, 38), in his extensive investigations of paunch fermentation in cattle, found in nearly every instance a small amount of hydrogen, and Von der Heide, Klein, and Zuntz (20), in respiration experiments upon an ox, observed a small excretion of hydrogen in two cases out of four. In computing the energy losses in the following experiments it has been assumed that the combustible gases consisted of CH_4 (methane), and the computations have been based upon the observed quantity of carbon.

The difference between the chemical energy of the feed and that lost in the excreta shows how much of the former is capable of being converted into other forms in the body, either during the changes which the feed undergoes in the digestive tract or in the course of metabolism in the tissues. This convertible portion of the feed energy has been given various names by different investigators, such as "physiological heat value," "fuel value," "available energy," etc. Without entering here into a discussion of the propriety of these names, we have preferred for our present purpose to follow the suggestion made earlier by the senior author (2, p. 270) and to designate it as "metabolizable energy." By this term is meant simply the energy capable of transformation in the body, with no implications as to the proportion of the energy thus trans-

formed which can be utilized by the organism. The heat evolved during the methane fermentation, for example, constitutes part of the metabolizable energy as thus defined, although it does not enter into the tissue metabolism.

The determination of the losses of chemical energy from a single feeding stuff or from a mixed ration is relatively simple, as is illustrated by the following example taken from the results on steer B in experiment 207.

Computation of losses of chemical energy from a ration

	Period 2.		Period 3.	
Energy of feed:				
Timothy hay	12,477 Cals.		12,618 Cals.	
Grain mixture No. 1	12,549 Cals.		.....	
Total		25,026 Cals.		12,618 Cals.
Energy of excreta:				
Feces	7,371 Cals.		5,247 Cals.	
Urine	1,536 Cals.		627 Cals.	
Methane	2,098 Cals.		1,057 Cals.	
Total		11,005 Cals.		6,931 Cals.
Metabolizable energy		14,021 Cals.		5,687 Cals.

Since concentrates can not be fed alone, the losses of chemical energy which they suffer, like their digestibility, must be obtained by means of a calculation by difference, which in period 2 of the foregoing example is as follows:

Computation of losses of chemical energy by a concentrate

	Chemical energy of feed. Calories.	Chemical energy of excreta.			Metabolizable energy. Calories.
		Feces. Calories.	Urine. Calories.	Methane. Calories.	
Total ration	25,026	7,371	1,536	2,098	14,021
Computed for hay	12,477	5,254	591	1,003	5,629
Grain mixture by difference	12,549	2,117	945	1,095	8,392

Computed in the manner just illustrated, the losses of chemical energy per kilogram of dry matter consumed in these experiments and the metabolizable energy remaining are shown in Table III, which includes also the percentage distribution of the feed energy between the various excreta, on the one hand, and the metabolizable energy, on the other.[1] For convenience, the average results for the metabolizable energy per kilogram of dry matter and per kilogram of digestible organic matter are brought together in Table IV.

[1] In all cases the observed energy of the urine has been corrected to nitrogen equilibrium of the animal by adding 7.5 Calories for each gram of nitrogen retained by the animal or subtracting the same amount for each gram of body nitrogen lost, the correction being regarded as representing energy of excretory material temporarily retained in the body.

TABLE III.—*Losses of energy and their percentage distribution*

Feeding stuff and experiment No.	Animal No.	Period No.	Dry matter eaten per day and head.		Energy per kilogram of dry matter.					Metabolizable per kilogram of digestible organic matter.	Percentage losses.			Percentage metabolizable.
			Coarse feed.	Concentrates.	Total.[a]	Losses.			Metabolizable.		In feces.	In urine.	In CH_4.	
						In feces.	In urine.[b]	In CH_4.						
Timothy hay:			*Gm.*	*Gm.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*				
174[c]	I	D–A	4,483	356	4,554	2,439	156	285	1,674	3,483	53.56	3.44	6.25	36.75
190	A	3	2,001	.....	4,502	2,209	164	309	1,820	3,429	49.06	3.64	6.87	40.43
190	A	4	3,493	.....	4,488	2,306	128	303	1,751	3,480	51.38	2.86	6.75	39.01
190	B	3	1,774	.....	4,503	2,209	141	342	1,811	3,399	49.07	3.14	7.60	40.20
190	B	4	2,610	.....	4,483	2,176	125	304	1,878	3,484	48.55	2.79	6.78	41.88
Average	....	.....	2,470	.....	4,494	2,225	139	315	1,815	3,448	49.51	3.09	7.01	40.39
200	A	3	2,647	.....	4,510	2,175	155	[b] 314	1,866	3,597	48.22	3.44	6.97	41.37
200	A	4	4,425	.....	4,509	2,250	153	[b] 301	1,805	3,566	49.91	3.40	6.68	40.01
200	B	3	2,470	.....	4,510	2,092	168	[b] 326	1,924	3,565	46.39	3.72	7.24	42.65
200	B	4	3,805	.....	4,509	2,164	167	[b] 312	1,866	3,565	48.00	3.70	6.92	41.38
Average	....	.....	.....	.....	4,509	2,170	161	313	1,865	3,573	48.13	3.57	6.94	41.36
207	A	3	2,974	.....	4,509	1,841	204	368	2,096	3,457	40.82	4.52	8.17	46.49
207	A	4	4,892	.....	4,521	1,888	199	357	2,077	3,482	41.77	4.40	7.90	45.93
207	B	3	2,798	.....	4,509	1,875	224	378	2,032	3,378	41.58	4.97	8.38	45.07
207	B	4	4,630	.....	4,521	1,918	208	355	2,040	3,456	42.42	4.61	7.84	45.13
Average	....	.....	.....	.....	4,513	1,880	208	364	2,061	3,443	41.66	4.61	8.07	45.66
Red-clover hay:														
179	I	1	4,459	.....	4,450	1,940	306	309	1,895	3,373	43.61	6.87	6.94	42.58
179	I	2	3,144	.....	4,426	1,857	288	324	1,957	3,452	41.95	6.50	7.33	44.22
Average	....	.....	.....	.....	4,438	1,898	297	317	1,926	3,413	42.77	6.69	7.14	43.40
186	I	1a	2,933	.....	4,490	1,842	326	303	2,019	3,460	41.03	7.25	6.75	44.97
186	I	2a	5,025	.....	4,489	1,817	301	243	2,129	3,637	40.49	6.72	5.41	47.38
186	I	3a	4,139	.....	4,478	1,852	290	254	2,082	3,578	41.36	6.48	5.68	46.48
Average	....	.....	.....	.....	4,486	1,837	306	267	2,076	3,558	40.95	6.82	5.95	46.28
Mixed hay:														
211	D	1	6,204	.....	4,400	2,077	209	285	1,829	3,442	47.20	4.75	6.49	41.56
211	D	4	3,498	.....	4,391	1,837	234	332	1,990	3,406	41.83	5.33	7.54	45.30
211	D	5	1,786	.....	4,390	1,956	255	345	1,834	3,294	44.57	5.80	7.85	41.78
211	G	1	6,092	.....	4,393	1,906	208	300	1,979	3,463	43.39	4.75	6.82	45.04
211	G	4	3,149	.....	4,391	1,878	220	319	1,974	3,420	42.76	5.00	7.27	44.97
211	G	5	1,608	.....	4,390	1,917	238	358	1,877	3,313	43.68	5.41	8.15	42.76
Average	....	.....	.....	.....	4,393	1,929	227	323	1,914	3,390	43.92	5.17	7.35	43.56
Alfalfa hay:														
208	D	1	2,155	.....	4,407	2,124	244	262	1,777	3,529	48.21	5.54	5.93	40.32
208	D	2	1,300	.....	4,407	2,186	266	273	1,682	3,437	49.61	6.03	6.17	38.19
208	E	4	4,170	.....	4,410	2,043	231	241	1,895	3,697	46.33	5.24	5.46	42.97
208	E	5	2,408	.....	4,407	2,105	249	276	1,777	3,518	47.78	5.64	6.26	40.32
208	E	6	1,413	.....	4,406	2,037	248	281	1,840	3,528	46.24	5.63	6.37	41.76
208	C	4	4,744	.....	4,403	2,184	233	218	1,768	3,655	49.61	5.29	4.95	40.15
208	C	5	3,099	.....	4,407	2,066	243	270	1,828	3,594	46.90	5.51	6.12	41.47
208	C	6	2,119	.....	4,406	2,013	250	280	1,863	3,576	45.69	5.68	6.37	42.26
Average	....	.....	.....	.....	4,407	2,095	246	262	1,804	3,567	47.54	5.58	5.94	40.94

[a] From analyses of individual samples for each period, and therefore differ somewhat from the averages of Table I.
[b] Corrected to N equilibrium, using Rubner's factor, 7.45 Calories per gram N.
[c] Computed by difference.

TABLE III.—*Losses of energy and their percentage distribution*—Continued

Feeding stuff and experiment No.	Animal No.	Period No.	Dry matter eaten per day and head.		Energy per kilogram of dry matter.					Metabolizable per kilogram of digestible organic matter.	Percentage losses.			Percentage metabolizable.
			Coarse feed.	Concentrates.	Total.	Losses.			Metabolizable.		In feces.	In urine.	In CH_4.	
						In feces.	In urine.	In CH_4.						
Alfalfa hay—Con.			*Gm.*	*Gm.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*				
209	F	4	6,174	.....	4,359	2,046	218	248	1,847	3,652	46.95	5.00	5.69	42.36
209	F	5	3,562	.....	4,328	1,961	244	264	1,859	3,588	45.31	5.63	6.11	42.96
209	F	6	2,226	.....	4,328	2,078	246	281	1,723	3,470	48.01	5.67	6.49	39.83
Average	....	.....	.....	.....	4,338	2,028	236	264	1,810	3,570	46.75	5.44	6.09	41.72
212	H	1	6,638	.....	4,354	1,796	275	277	2,006	3,550	41.26	6.31	6.37	46.06
212	H	3	5,320	.....	4,338	1,805	270	283	1,980	3,525	41.60	6.23	6.53	45.64
212	H	5	3,052	.....	4,413	1,642	290	299	2,181	3,729	37.21	6.58	6.78	49.43
Average	....	.....	.....	.....	4,368	1,748	278	286	2,056	3,601	40.02	6.36	6.55	47.07
Alfalfa meal:														
212	H	2	6,671	.....	4,364	1,889	252	250	1,973	3,671	43.29	5.78	5.74	45.19
212	H	4	5,408	.....	4,379	1,897	250	259	1,973	3,646	43.31	5.72	5.93	45.04
212	H	6	3,155	.....	4,379	1,726	270	292	2,091	3,727	39.41	6.18	6.66	47.75
Average	....	.....	.....	.....	4,374	1,838	257	267	2,012	3,681	42.01	5.89	6.11	45.99
Maize stover:														
210	D	1	4,335	.....	4,333	1,860	172	335	1,966	3,488	42.93	3.96	7.74	45.37
210	D	2	3,548	.....	4,327	1,837	185	335	1,970	3,493	42.47	4.27	7.75	45.51
210	D	3	2,563	.....	4,337	1,867	195	354	1,921	3,369	43.06	4.50	8.15	44.29
Average	....	.....	.....	.....	4,332	1,855	184	341	1,952	3,450	42.82	4.24	7.88	45.06
Maize meal:														
179	I	3	3,163	735	4,360	633	441	488	2,798	3,186	14.52	10.12	11.20	64.16
179	I	4	3,186	3,451	4,366	401	167	406	3,392	3,716	9.18	3.83	9.31	77.68
Average	....	.....	.....	.....	4,363	517	304	447	3,095	3,451	11.85	6.97	10.25	70.93
211	G	2	790	1,542	4,526	382	175	509	3,460	3,825	8.45	3.87	11.25	76.43
211	G	3	2,383	4,644	4,508	948	125	372	3,063	3,928	21.04	2.78	8.25	67.93
Average	....	.....	.....	.....	4,517	665	150	441	3,261	3,877	14.74	3.32	9.75	72.19
Wheat bran:														
190	A	1	1,978	1,370	4,545	1,484	230	365	2,466	4,003	32.67	5.06	8.04	54.24
190	A	2	1,996	2,583	4,558	1,440	221	311	2,586	3,982	31.59	4.85	6.82	56.74
190	B	1	1,712	999	4,563	1,500	254	332	2,477	3,951	32.87	5.56	7.28	54.29
190	B	2	1,779	1,803	4,461	1,336	271	340	2,514	3,872	29.96	6.07	7.62	56.35
Average	....	.....	.....	.....	4,532	1,440	244	337	2,511	3,954	31.77	5.38	7.44	55.41
Grain mixture No. 1:														
200	A	1	2,608	1,792	4,698	611	358	[a] 390	3,339	3,946	13.00	7.62	8.30	71.08
200	A	2	2,631	4,173	4,695	1,051	288	[a] 358	2,998	3,974	22.38	6.13	7.63	63.86
200	B	1	2,433	1,195	4,697	639	385	[a] 381	3,294	3,923	13.60	8.19	8.11	70.10
200	B	2	2,454	2,129	4,695	1,064	322	[a] 343	2,966	3,998	22.66	6.86	7.30	63.18
Average	....	.....	.....	.....	4,696	841	338	368	3,149	3,960	17.91	7.20	7.84	67.05
207	A	1	2,935	1,996	4,658	951	316	397	2,994	3,876	20.42	6.78	8.52	64.28
207	A	2	2,949	4,759	4,688	1,019	294	342	3,033	3,991	21.73	6.28	7.29	64.70
207	B	1	2,761	1,398	4,658	1,104	363	439	2,752	3,683	23.71	7.79	9.42	59.08
207	B	2	2,774	2,677	4,688	791	353	409	3,135	3,888	16.87	7.53	8.72	66.88
Average	....	.....	.....	.....	4,673	966	332	397	2,978	3,860	20.68	7.09	8.48	63.75

[a] Computed from digestible carbohydrates.

TABLE III.—*Losses of energy and their percentage distribution*—Continued

Feeding stuff and experiment No.	Animal No.	Period No.	Dry matter eaten per day and head.		Energy per kilogram of dry matter.					Metabolizable per kilogram of digestible organic matter.	Percentage losses.			Percentage metabolizable.
			Coarse feed.	Concentrates.	Total.	Losses.			Metabolizable.		In feces.	In urine.	In CH_4.	
						In feces.	In urine.	In CH_4.						
Grain mixture No. 2:			*Gm.*	*Gm.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*				
208.	F	1	1,086	2,122	4,607	1,258	173	349	2,827	3,887	27.32	3.75	7.58	61.36
208.....	E	2	590	1,163	4,611	840	202	442	3,127	3,837	18.22	4.38	9.57	67.83
208.....	E	3	387	764	4,595	901	236	346	3,112	3,890	19.61	5.14	7.54	67.72
208.....	C	2	731	1,453	4,611	1,246	192	463	2,710	3,706	27.03	4.16	10.04	58.77
208.....	C	3	521	1,018	4,594	826	242	486	3,038	3,711	17.98	5.28	10.58	66.16
Average..	....	.....	.....	.....	4,604	1,014	209	417	2,964	3,806	22.03	4.54	9.06	64.37
209.....	F	1	1,462	3,040	4,629	1,312	170	283	2,864	3,979	28.35	3.68	6.11	61.86
209.....	F	2	894	1,862	4,605	1,032	184	327	3,062	3,980	22.40	3.99	7.10	66.51
209.....	F	3	536	1,112	4,604	891	223	376	3,114	3,896	19.36	4.85	8.16	67.63
Average..	....	.....	.....	.....	4,613	1,078	193	329	3,013	3,952	23.37	4.17	7.12	65.34
Hominy chop:														
211.....	D	2	1,747	1,764	4,720	542	195	496	3,487	3,993	11.48	4.13	10.50	73.89
211.....	D	3	3,910	3,949	4,698	603	167	371	3,557	4,156	12.83	3.56	7.90	75.71
Average..	....	.....	.....	.....	4,709	573	181	433	3,522	4,075	12.15	3.84	9.20	74.81
Alfalfa hay and grain mixture No. 2:														
208.....	E	1	1,086	2,122	4,531	1,526	195	318	2,492	3,810	33.69	4.30	7.01	55.00
208.....	E	2	590	1,163	4,535	1,247	214	380	2,694	3,777	27.50	4.72	8.37	59.41
208.....	E	3	387	764	4,532	1,291	237	316	2,688	3,824	28.49	5.23	6.98	59.30
208.....	C	2	731	1,453	4,543	1,536	208	390	2,409	3,687	33.81	4.58	8.61	53.00
208.....	C	3	521	1,018	4,536	1,264	242	406	2,624	3,696	27.86	5.33	8.95	57.86
Average..	....	.....	.....	.....	4,535	1,373	219	362	2,581	3,759	30.27	4.83	7.98	56.92
209.....	F	1	1,462	3,040	4,527	1,540	189	275	2,523	3,872	34.67	4.26	6.18	54.89
209.....	F	2	894	1,862	4,516	1,352	199	305	2,660	3,888	29.94	4.40	6.74	58.92
209.....	F	3	536	1,112	4,515	1,259	225	338	2,693	3,826	27.88	4.99	7.48	59.65
Average..	....	.....	.....	.....	4,519	1,384	204	306	2,625	3,862	30.62	4.51	6.77	58.09
Mixed hay and maize meal:														
211.....	G	2	790	1,542	4,483	897	189	443	2,954	3,701	20.00	4.22	9.88	65.90
211.....	G	3	2,383	4,644	4,468	1,271	156	352	2,689	3,763	28.45	3.49	7.88	60.18
Average..	....	.....	.....	.....	4,476	1,084	173	398	2,821	3,732	24.22	3.87	8.89	63.02
Mixed hay and hominy chop:														
211.....	D	2	1,747	1,764	4,560	1,261	209	403	2,687	3,775	27.65	4.58	8.84	58.93
211.....	D	3	3,910	3,949	4,553	1,293	196	340	2,724	3,879	28.40	4.30	7.47	59.83
Average..	....	.....	.....	.....	4,557	1,277	203	371	2,706	3,827	28.02	4.44	8.15	59.39

TABLE IV.—*Average losses of chemical energy—metabolizable energy*

Kind of feed and experiment No.	Gross energy per kilogram of dry matter.	Losses of chemical energy per kilogram of dry matter.	Metabolizable energy.	
			Per kilogram of dry matter.	Per kilogram of digestible organic matter.
Timothy hay:	*Calories.*	*Calories.*	*Calories.*	*Calories.*
174	4,554	2,880	1,674	3,483
190	4,494	2,679	1,815	3,448
200	4,509	2,644	1,865	3,573
207	4,513	2,452	2,061	3,443
Average	4,518	2,664	1,854	3,487
Red clover hay:				
179	4,438	2,512	1,926	3,413
186	4,486	2,410	2,076	3,558
Average	4,462	2,461	2,001	3,486
Mixed hay:				
211	4,393	2,479	1,914	3,390
Alfalfa hay:				
208	4,407	2,603	1,804	3,567
209	4,338	2,528	1,810	3,570
212	4,368	2,312	2,056	3,601
Average	4,371	2,481	1,890	3,579
Alfalfa meal:				
212	4,374	2,362	2,012	3,681
Average of alfalfa hay and meal	4,372	2,451	1,921	3,605
Maize stover:				
210	4,332	2,380	1,952	3,450
Maize meal:				
179[a]	4,366	974	3,392	3,716
211	4,517	1,256	3,261	3,877
Average	4,442	1,115	3,327	3,797
Wheat bran:				
190	4,532	2,021	2,511	3,954
Grain mixture No. 1:				
200	4,696	1,547	3,149	3,960
207	4,673	1,695	2,978	3,860
Average	4,685	1,621	3,064	3,910
Grain mixture No. 2:				
208	4,604	1,640	2,964	3,806
209	4,613	1,600	3,013	3,952
Average	4,609	1,620	2,989	3,879
Hominy chop:				
211	4,709	1,187	3,522	4,075

[a] Period 4.

The results recorded in Table III illustrate the familiar fact that the greatest loss of chemical energy, especially in the case of coarse feeds, is that in the undigested feed residues of the feces and in the relatively small amounts of excretory products which, in the case of cattle, accompany them. The relative proportions lost in the urine and in the methane naturally vary with the composition of the feed, one rich in protein tending especially to increase the energy content of the urine, while carbohydrates tend to increase the excretion of methane.

Of greater interest, however, is the variability of the losses suffered by the same feeding stuff in different periods.

INFLUENCE OF QUANTITY OF FEED CONSUMED ON LOSSES OF CHEMICAL ENERGY

In considering this question it should be borne in mind that the comparisons here reported are in every instance between *different amounts of the same ration*—i. e., they deal with the influence of quantity only and do not touch the question of the influence of heavy grain feeding. Furthermore, they relate to comparatively light feeding, many of the periods having been upon submaintenance rations, while the total dry matter of the feed seldom reached 18 pounds per 1,000 pounds of live weight. The experiments recorded in Table III include 31 cases in which different amounts either of a single feeding stuff or of an identical mixed ration were consumed by the same animal in two different periods of the same experiment, under conditions as nearly uniform as it was possible to make them. The results may be most conveniently compared on the basis of the percentage distribution of the energy, as shown in the last four columns of the table. In the following comparisons the results computed by difference for the concentrated feeds are not included.

LOSSES IN METHANE

In a single instance (alfalfa hay and grain mixture No. 2 in experiment 208, steer E, periods 2 and 3) the percentage loss in the methane was greater on the heavier of the two rations and in another case (corn stover in experiment 210, steer D, periods 1 and 2) the difference was only 0.01 per cent. In two cases the determinations of methane are believed to have been inaccurate. In the remaining 29 cases the percentage loss in methane was distinctly greater on the lighter ration, the difference ranging from 0.11 to 2 per cent and tending, on the whole, to be somewhat greater on the mixed rations, with their larger proportion of readily soluble carbohydrates, than on those consisting exclusively of coarse fodder. In other words, as would be anticipated, the bacterial fermentation of the carbohydrates in the digestive tract of cattle proceeds to a distinctly greater extent on light than on heavy rations.

LOSSES IN URINE

The percentage of the feed energy excreted in the urine was also greater on the lighter ration in 28 cases out of 33, the exceptions being two experiments on alfalfa hay (experiment 208, steer E, periods 5 and 6,

and experiment 212, steer H, periods 1 and 3), two on clover hay (experiment 179, steer I, periods 1 and 2, and experiment 186, steer I, periods 2a and 3a), and one on alfalfa meal (experiment 212, steer H, periods 2 and 4). In the remaining 28 cases there is one in which the difference amounts to only 0.02 per cent and two in which it is 0.04 per cent. In the remaining 25 it ranges from 0.12 to 0.78 per cent. This greater relative loss in the urine on the lighter ration can not be attributed to the presence of nitrogenous substances derived from an increased katabolism of body protein, since the energy of the urine has been at least approximately corrected to nitrogen equilibrium (p. 440). Since it is well established that the urine of cattle contains a considerable quantity of nonnitrogenous substances (2, p. 312–314, 320–322), it seems not impossible that the more extensive fermentation on the lighter ration may have resulted in an increase of these unknown constituents.

LOSSES IN FECES

The results regarding the losses of chemical energy in the feces are by no means so uniform as in the case of the methane and of the urine. In 22 out of 33 cases there is a distinctly smaller relative loss of energy in the feces with the lighter ration—i. e., a greater apparent digestibility—the difference in the percentages ranging from 0.28 to 8.45. In the other third of the cases, however, the difference is in the opposite direction, ranging from 0.37 to 2.74, with the exception of one case of practical equality, so that it appears that other factors besides the extent of the methane fermentation affected the percentage digestibility. Two rather marked cases of a greater loss of energy in the feces on the lighter ration are found in experiment 211, periods 4 and 5, with relatively very small rations. Whether the relative loss in the feces increases or decreases with an increase of the ration seems to bear no relation to the total quantity of feed consumed either per head or per 500 kg. of live weight. The 10 instances in which a greater percentage loss in the feces was observed on the lighter of the two rations include, it is true, the more extreme rations as regards the total quantity, but the averages for the two groups are not widely different (4,376 and 3,952 gm. of dry matter).

PERCENTAGE OF FEED ENERGY METABOLIZABLE

The bearing of the foregoing facts upon the percentage of the feed energy which is metabolizable is obvious. Clearly the fermentation which plays so large a rôle in the digestive processes of ruminants was relatively more intense on the lighter rations, resulting in the breaking down of a larger proportion of the carbohydrates and in a greater loss of chemical energy in the methane, accompanied in most instances by an increased loss in the urine also. On the other hand, however, the organic acids resulting from the fermentation are resorbed and oxidized in the body, and their energy, together with the heat evolved in the fermentation, constitutes part of the metabolizable energy of the feed as defined on page 439. Whether the proportion of the total energy of the feed which is metabolizable be greater or less on the lighter ration will depend, therefore, upon the nature of the additional carbohydrates fermented. If the increased fermentation attacks the more insoluble carbohydrates, which would otherwise escape digestion entirely and

reappear in the feces, the result will be a relative increase in the metabolizable energy. On the other hand, if the greater fermentation on the lighter rations is at the expense of the more soluble carbohydrates which would otherwise be digested by the enzyms of the intestines, the metabolizable energy will be diminished by the quantity of chemical energy escaping in the methane (and urine). It is perhaps not surprising, therefore, to find that the effect of the quantity of feed upon the percentage of energy metabolizable was somewhat variable.

In 19 out of 22 cases mentioned in the previous paragraph in which the percentage loss of energy in the feces decreased as the amount of feed consumed was diminished, the percentage of energy metabolized did in fact increase, while in the remaining 3 cases the greater losses in methane and urine overbalanced the effect of the increased digestibility. In each of the 10 instances in which the percentage loss of energy in the feces was greater on the lighter rations, the percentage metabolizable shows a corresponding decrease, so that of the entire 33 comparisons 14 show a greater and 19 a less percentage metabolizable on lighter as compared with heavier rations.

On the whole, then, the differences in amount of feed consumed, within the limits of these experiments, failed to show any unmistakable effect upon the quantity of energy actually liberated in the body from a unit weight of feed. Morever, it must be borne in mind that a considerable part of the additional energy secured by the more extensive fermentation of the lighter ration is liberated in the digestive tract as heat of fermentation and does not enter into the energy exchange of the body tissues, so that the difference in the net nutritive effect is likely to be less than that in the metabolizable energy as ordinarily defined.

INFLUENCE OF INDIVIDUALITY ON LOSSES OF CHEMICAL ENERGY

In five of the experiments the same feeding stuff or mixture of feeding stuffs was fed to more than one animal, although unfortunately the amounts consumed were not the same either per head or in proportion to the live weight, so that exact comparisons are not possible.

In experiments 190, 200, and 207 a pure-bred Shorthorn steer was compared with a so-called scrub. A comparison of the averages for the lighter and the heavier rations of timothy hay, respectively, for the three successive years gives the averages shown in Table V, which fail to show any distinct individual difference between the two animals. The results computed by difference for the wheat bran in experiment 190 and for the grain mixture No. 1 in experiments 200 and 207 show somewhat larger numerical differences, but when the errors incident to such calculations by difference are considered they agree with those upon hay in showing no material difference between these two animals. This point has been discussed from a slightly different standpoint elsewhere (10).

TABLE V.—*Influence of individuality of cattle on losses of chemical energy*

Character of experiment.	Loss of energy.			Metabolizable.
	In feces.	In urine.	In methane.	
Timothy hay (experiments 190, 200, and 207):				
Average of light rations—	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Steer A	46.03	3.87	7.34	42.76
Steer B	46.03	3.96	7.79	42.22
Difference	0	− .09	− .45	+ .54
Average of heavy rations—				
Steer A	47.69	3.55	7.11	41.65
Steer B	46.32	3.70	7.18	42.80
Difference	+ 1.37	− .15	− .07	− 1.15
Average of all rations—				
Steer A	46.86	3.71	7.22	42.21
Steer B	46.18	3.83	7.49	42.50
Difference	+ .68	− .12	− .27	− .29
Alfalfa hay (experiment 208):				
Average of medium and light rations—				
Steer D (periods 1 and 2)	48.91	5.79	6.05	39.25
Steer E (periods 5 and 6)	47.01	5.64	6.31	41.04
Steer C (periods 5 and 6)	46.30	5.60	6.24	41.86
Difference (D−C)	+ 2.61	+ .19	− .19	− 2.61
Heavy rations—				
Steer E (period 4)	46.33	5.24	5.46	42.97
Steer C (period 4)	49.61	5.29	4.95	40.15
Difference	− 3.28	− .05	+ .51	+ 2.82
Alfalfa hay and grain (experiment 208):				
Average of medium and light rations—				
Steer E (periods 2 and 3)	28.00	4.98	7.67	59.35
Steer C (periods 2 and 3)	30.83	4.96	8.78	55.43
Difference	− 2.83	+ .02	−1.11	+ 3.92
Mixed hay (experiment 211):				
Steer D (periods 1, 4, and 5)	44.53	5.29	7.30	42.88
Steer G (periods 1, 4, and 5)	43.28	5.05	7.41	44.26
Difference	+ 1.25	+ .24	− .11	− 1.38

The three animals used in experiment 208 had been subjected to different previous treatment, steer D having received almost from birth a restricted quantity of feed, steer E a ration ample to support normal growth, and steer C as heavy feeding as practicable. In the periods on the medium and light rations of alfalfa hay these animals showed slight differences in their digestive powers in the order named, the average

results for the two quantities being as shown in Table V. On the other hand, upon the heavy hay ration and also upon the mixed ration of hay and grain steer C was distinctly inferior to steer E, losing more chemical energy in both methane and feces. This was the most distinct individual difference in these experiments. It should perhaps be noted that steer C showed a tendency to bloat and to get off feed on heavy rations and possibly did not have full normal digestive power. In experiment 211 steer D, then a year older, on the average of three periods on mixed hay again showed a slight inferiority to another animal which presumably had received better feeding during growth.

Clearly individual differences between the animals had no very material influence on the losses of chemical energy in these experiments. In most instances the differences are well within the limits of error for such determinations, and even in those cases where there seem to be distinct individual differences they are comparatively slight, being of about the same magnitude as those observed by G. Kühn (28) and rather smaller than those found by Armsby (1) in experiments on three steers.

VARIABILITY OF METABOLIZABLE ENERGY

The results recorded in Tables III and IV show clearly that the metabolizable energy of a feeding stuff is by no means a constant. Not only do the averages for feeding stuffs of the same name differ more or less, but the metabolizable energy of the same sample is more or less variable in the different periods.

The losses of chemical energy which a feeding stuff suffers are substantially determined by the nature and extent of the digestive processes. Digestibility, however, especially in ruminants, is a very complex affair, depending on many factors. Broadly speaking, it may be characterized as a series of fermentations, effected in part by a variety of organized ferments and in part by enzyms secreted by the digestive organs or contained in the feed itself. Changes in the composition of the contents of the digestive tract or in the rapidity with which they move forward through it can hardly fail to influence in a variety of ways the course of these fermentations, and it seems on the whole rather surprising that they go forward as uniformly as they do.

In these experiments they appear to have been affected chiefly by the variations in the amount of feed consumed. Recently Zuntz and his associates (20, 53) have reported striking instances in which the extent of the methane fermentation in particular has been markedly affected by the make-up of the rations and especially by the order in which the feeds were consumed, while Völtz and his associates (47, 48) have laid much stress on the practical importance of these results. No such marked differences occurred in our experiments, but, on the other hand, the range of feeds was not so wide. It is perhaps too early

to judge the full significance of Zuntz's results, but they should at least serve to correct the notion, more or less subconsciously held by not a few, of digestion as a perfectly definite process and of a digestion coefficient as a sort of chemical constant. On the other hand, however, it is easy to overestimate the importance of these variations in the digestive process in their bearing upon estimates of the values of feeding stuffs. On the whole, they appear to be of far less significance than other factors to be considered later.

ESTIMATION OF METABOLIZABLE ENERGY FROM METABOLISM EXPERIMENTS

The losses of chemical energy in feces and urine are readily determined by means of the ordinary metabolism experiment, but the determination of the losses in the combustible gases requires special and somewhat costly apparatus. A number of experimenters have therefore attempted to estimate the amounts of these gases produced, usually from the amounts of carbohydrates (crude fiber and nitrogen-free extract) digested, using, as a rule, the average factor derived from Kellner's investigations (26, p. 420)—viz, 4.2 parts of CH_4 per 100 parts of digested carbohydrates.

Our experiments have yielded somewhat higher figures, as shown in Table VI, giving the maximum, minimum, and average results for each feeding stuff or mixture.

TABLE VI.—*Quantity of methane per 100 gm. of digestible carbohydrates*

Feeding stuff.	Number of experiments.	Quantity of methane.		
		Maximum.	Minimum.	Average.
		Gm.	*Gm.*	*Gm.*
Timothy hay	12	3.8	5.1	4.6
Clover hay	5	3.9	5.2	4.6
Mixed hay	6	4.6	5.8	5.1
Alfalfa hay	17	4.2	5.3	4.8
Maize stover	3	4.7	4.8	4.7
Average	43	.....	.....	4.8
Maize meal and clover hay	2	4.3	5.2	4.8
Wheat bran and timothy hay	4	4.8	5.2	4.9
Grain mixture No. 1 and timothy hay	4	4.7	5.3	5.0
Grain mixture No. 2 and alfalfa hay	8	3.8	5.5	4.5
Maize meal and mixed hay	2	4.2	4.7	4.5
Hominy chop and mixed hay	2	4.4	5.0	4.7
Average	22	.....	.....	4.7
Average of author's experiments	65	3.8	5.5	4.8
Average of Kellner's experiments	44	2.9	5.5	4.2

While there is a considerable range in the results of individual experiments, nevertheless an estimate of 4.5 grams of CH_4 per 100 gm. of digested carbohydrates affords a fair basis for an approximate estimate of tne losses of chemical energy in the combustible gases and for computing the metabolizable energy of a feeding stuff by means of the ordinary digestion experiment combined with the collection of the urine and a determination of the heats of combustion of the visible excreta. The additional labor required for this purpose is so small that it is to be hoped that in future digestion experiments it may be undertaken whenever possible.

FROM DIGESTIBLE ORGANIC MATTER

It is possible also to estimate the average metabolizable energy of a feeding stuff from its content of total digestible organic matter, as shown by the ordinary feeding tables.

The differences shown in Tables III and IV between the metabolizable energy per kilogram of dry matter of the different feeding stuffs are due chiefly, as already pointed out, to differences in the proportion of the chemical energy carried off in the feces, so that the metabolizable energy per kilogram of digestible organic matter shows a striking degree of uniformity as between different coarse feeds, on one hand, and as between different concentrates, on the other, a fact quite in harmony with earlier results reported by Kellner (26). Expressing the results in therms per kilogram and using for the apparent metabolizable energy of Kellner and Köhler's feeding stuffs the figures computed by Armsby (2, p. 301), we obtain the following averages:

Metabolizable energy per kilogram of digestible organic matter

COARSE FEEDS

	Therms.
Armsby and Fries:	
Timothy hay	3.49
Red clover hay	3.49
Mixed hay	3.39
Alfalfa hay and meal	3.61
Maize stover	3.45
Average	3.48
Kellner and Köhler:	
Meadow hay	3.50
Oat straw	3.74
Wheat straw	3.31
Extracted straw	3.64
Average	3.55

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CONCENTRATES

	Therms.
Armsby and Fries:	
Maize meal	3.80
Wheat bran	3.99
Grain mixture No. 2	3.88
Average	3.89
Grain mixture No. 1	3.91
Hominy chop	4.08
Average	4.00
Kellner and Köhler:	
Beet molasses	3.47
Starch	3.60
Wheat gluten	4.79

Tangl (44), Tangl and Weiser (45), and Zaitschek (50) have also determined the metabolizable energy of a number of feeding stuffs for cattle, the methane being estimated from the amount of digestible carbohydrates, with the following results, which are very similar to those just reported:

Metabolizable energy per kilogram of digestible organic matter: Tangl's experiments

	Therms.
Meadow hay	3.44
Ensiled meadow hay	3.70
Hay from irrigated meadows	3.60
Broom-corn millet meal	3.68
Pumpkins	4.29

On the other hand, four experiments upon a bull by Völtz et al. (48), in which the production of methane was likewise computed, gave notably higher figures, viz:

Metabolizable energy per kilogram of digestible organic matter: Völtz's experiments

	Therms.
Mixed ration (hay, straw, malt sprouts, dried brewers' grains, and potato flakes)	3.95
Dried distillery residue (from potatoes)	4.84
Palm-nut meal	4.85
Beet molasses	4.36

The most important factor influencing the metabolizable energy of the digestible organic matter of concentrates seems to be the percentage of fat in the feeding stuff, as appears from a comparison of the data contained in Table I, while feeding stuffs exceptionally high in protein have also a high content of metabolizable energy in their digestible matter, as in the case of Kellner and Köhler's wheat gluten. There seems no obvious explanation of the exceptionally high results obtained by Völtz, but it would seem that for the present, with the ordinary dry feeding stuffs or mixtures, the following factors may safely be made the basis for computing approximately the metabolizable energy of feeding stuffs for cattle when their content of digestible organic matter is known or can be estimated.

Metabolizable energy of feeding stuffs per kilogram of digestible organic matter

	Therms.
Coarse feeds	3.5
Concentrated feeds:	
With less than 5 per cent of digestible fat	3.9
With more than 5 per cent of digestible fat	4.0

No similar results have been reported on succulent feeds, with the exception of Zaitschek's figure for pumpkins.

II. EXPENDITURE OF ENERGY CONSEQUENT UPON FEED CONSUMPTION AND ITS FACTORS

That the consumption of feed tends to increase the metabolism of an animal has become a commonplace of physiology. The magnitude of the effect varies within rather wide limits according to the chemical and physical properties of the feed, while there is still more or less difference of opinion as to its causes. Zuntz and his associates have called it "work of digestion" and have attributed it largely to increased muscular and glandular activity of the digestive and excretory organs. Most physiological investigations in this field have been made on carnivora or on man, in which the increase of the metabolism is not usually very large, except when much protein is consumed. More recent investigations on these species appear to have shown that the mechanical work of the digestive organs is but a small factor and that the term "work of digestion" is not a fortunate one. With herbivora and especially with ruminants, however, the total effect on the metabolism is quantitatively very marked, while the mechanical factor is of much greater significance. This was early shown by Zuntz and Hagemann (52) in their investigations upon the horse. With ruminants the most extensive investigations are those made at the Möckern (Germany) Experiment Station by G. Kühn (29) and by Kellner (24, 25, 26, 27). These investigations, especially those of Kellner, were directed primarily to the determination of the relative values of nutrients and feeding stuffs, but from another point of view they constituted also determinations of the energy expenditure caused by the feed. The main purpose of the experiments here reported was to determine the proportion of the feed energy expended in the increased metabolism by means of direct measurements of the heat evolved, checked by determinations of the respiratory products, the relative values of the feeding stuffs being obtained by difference.

DIFFERENCES IN MUSCULAR ACTIVITY

INFLUENCE OF STANDING OR LYING UPON METABOLISM

We have repeatedly called attention to the very striking increase in the heat elimination of cattle when standing as compared with that when lying and have shown (11), in reply to the criticism of Zuntz, that it is

accompanied by a substantially corresponding increase in the gaseous excretion.

Since conclusions regarding the influence of feed consumption on the heat production must be drawn from comparisons of two or more periods on different amounts of feed, it is obvious that the periods must be made as nearly identical in other respects as practicable. It being impossible to control the standing and lying of the subject, it became necessary, therefore, to attempt a quantitative determination of the influence of standing upon the metabolism of the animal as measured by its heat production and on this basis to correct the results of each period to some uniform ratio of standing to lying. It was natural to suppose that the increment of the metabolism during standing was due to the work of supporting the body in an upright position, but it soon became evident that it was also to a very considerable extent a function of the feed consumption.

The apparatus used in these experiments is a flow calorimeter, the temperature difference between the ingoing and the outcoming water being read every four minutes. Since the hydrothermal equivalent of the absorber system and the contained water is only 6 kg., it is possible to follow very closely the rate of elimination of heat by radiation and conduction. The results found by the authors (11) show that the heat carried off as latent heat of water vapor is substantially proportional to that eliminated by radiation and conduction. On this basis the average heat elimination per minute and for 24 hours during standing and lying has been computed for each 48-hour period (or, in experiments 174, 190, 200, and 207, from selected uniform intervals). The addition of the necessary correction for the gain or loss of matter by the body gives the average heat production when standing or lying, respectively, while the difference between the two represents the increment due to standing for 24 hours. The following example, taken from experiment 179, period 1, in which 76.65 per cent of the total heat emitted was given off by radiation and conduction and 23.35 per cent as latent heat of water vapor, and in which the correction for loss of matter by the body was −127 Calories, may serve to illustrate the method.

Method of computing heat production of cattle when standing or lying

	Standing.	Lying.
Time standing or lying ..... minutes..	1, 767	1, 113
Heat emitted by radiation and conduction:		
Total ..... Calories..	12, 652	5, 128
Per minute ..... Calories..	7. 160	4. 607
Total heat production computed per 24 hours:		
Standing ..... 7. 160×1, 440÷0. 7665−127=	13, 324	Calories.
Lying ..... 4. 607×1, 440÷0. 7665−127=	8, 528	Calories.
Difference	4, 796	Calories.

The differences thus found between the heat production of the animals per 24 hours when standing and when lying were as shown in Table VII, in which are included for comparison the total dry matter consumed and the average live weight of the animal. In order to facilitate a general comparison of the results of the several experiments, the influence of the differences in live weight has also been eliminated to a degree by computing the data of Table V to a uniform body weight of 500 kg., with the results shown graphically in figure 1.

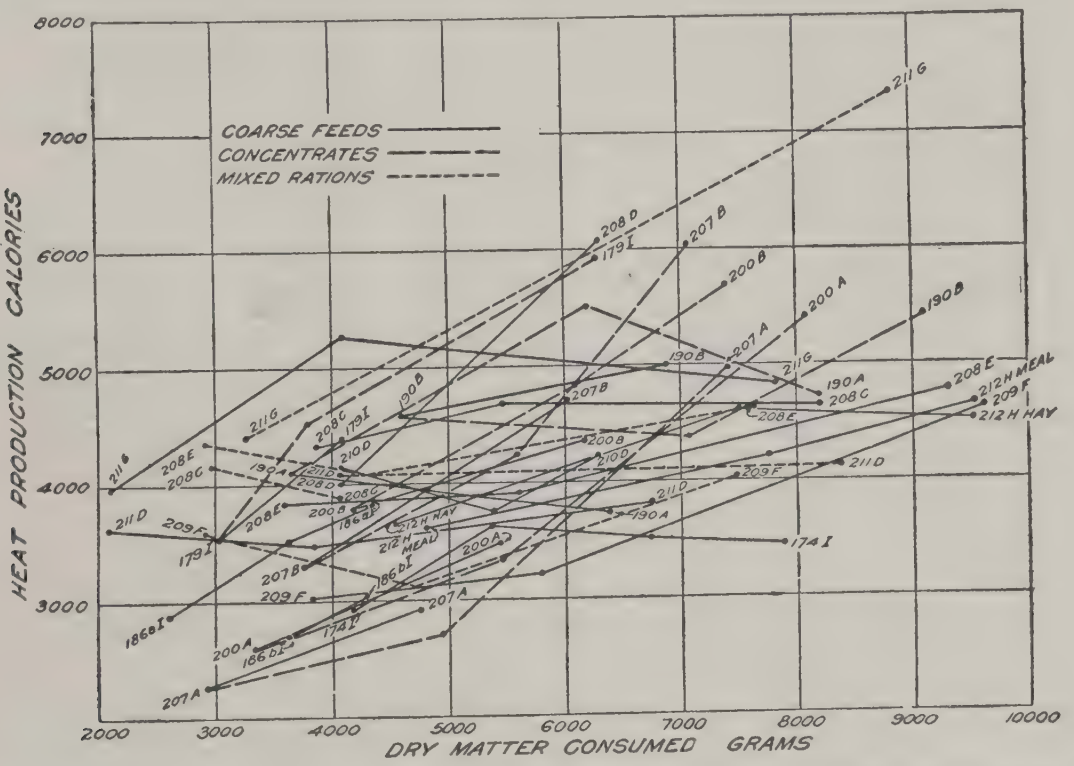

FIG. 1.—Graph showing the dry matter eaten and the increments of heat production due to standing; computed per 500 kg. live weight per 24 hours.

It should be clearly understood that both Table VII and figure 1 show the relation of feed consumption to the *heat increment during standing*, computed in the manner just illustrated, and not to the total heat production.

TABLE VII.—*Increments of heat production of cattle in standing*

Feeding stuff and experiment.	Average live weight.	Dry matter eaten per head.	Increment of heat production per 24 hours.	
			Per head.	Per kilogram of increment of dry matter eaten.
TIMOTHY HAY:				
Experiment 174—	*Kilograms.*	*Grams.*	*Calories.*	*Calories.*
Steer I (period A)	387	3,237	2,258	
Steer I (period B)	403	4,373	2,935	598
Steer I (period C)	416	5,480	2,913	−20
Steer I (period D)	424	6,440	2,928	16
Steer I (periods D–A)				210
Experiment 190—				
Steer A (period 3)	269	2,001	2,206	
Steer A (period 4)	278	3,493	2,076	−88
Steer B (period 3)	194	1,774	1,774	
Steer B (period 4)	190	2,610	1,899	148
Experiment 200—				
Steer A (period 3)	399	2,647	2,052	
Steer A (period 4)	407	4,425	2,838	442
Steer B (period 3)	296	2,470	2,233	
Steer B (period 4)	309	3,805	2,695	346
Experiment 207—				
Steer A (period 3)	507	2,974	2,293	
Steer A (period 4)	514	4,892	2,994	364
Steer B (period 3)	374	2,798	2,467	
Steer B (period 4)	385	4,630	3,618	628
RED CLOVER HAY:				
Experiment 179—				
Steer 1 (period 1)	545	4,459	4,794	
Steer 1 (period 2)	520	3,144	3,679	848
Experiment 186 (series *a*)—				
Steer 1 (period 1)	571	2,933	3,247	
Steer 1 (period 3)	580	4,139	4,018	637
Steer 1 (period 2)	587	5,025	4,438	474
Steer 1 (periods 2–1)				570
Experiment 186 (series *b*)—				
Steer 1 (period 3)	566	4,139	3,080	
Steer 1 (period 2)	576	5,025	3,452	420
MIXED HAY:				
Experiment 211—				
Steer D (period 1)	460	6,204	3,515	
Steer D (period 4)	455	3,498	3,147	136
Steer D (period 5)	428	1,786	3,107	24
Steer D (periods 1–5)				92
Steer G (period 1)	389	6,092	3,764	
Steer G (period 4)	387	3,149	4,066	−102
Steer G (period 5)	364	1,608	2,887	764
Steer G (periods 1–5)				196
ALFALFA HAY:				
Experiment 208—				
Steer D (period 1)	171	2,155	2,077	
Steer D (period 2)	162	1,300	1,314	894
Steer E (period 4)	224	4,170	2,145	
Steer E (period 5)	215	2,408	1,683	264
Steer E (period 6)	197	1,413	1,512	170
Steer E (periods 4–6)				230
Steer C (period 4)	289	4,744	2,690	
Steer C (period 5)	284	3,099	2,659	18
Steer C (period 6)	275	2,119	2,380	286
Steer C (periods 4–6)				118

TABLE VII.—*Increments of heat production of cattle in standing*—Continued

Feeding stuff and experiment.	Average live weight.	Dry matter eaten per head.	Increment of heat production per 24 hours.	
			Per head.	Per kilogram of increment of dry matter eaten.
ALFALFA HAY—Continued.				
Experiment 209—	*Kilograms.*	*Grams.*	*Calories.*	*Calories.*
Steer F (period 4)	321	6,174	2,973	372
Steer F (period 5)	308	3,562	1,997	172
Steer F (period 6)	292	2,226	1,768	
Steer F (periods 4–6)				304
Experiment 212—				
Steer H (period 1)	349	6,638	3,167	−44
Steer H (period 3)	354	5,320	3,224	328
Steer H (period 5)	337	3,052	2,481	
Steer H (periods 1–5)				192
ALFALFA MEAL:				
Experiment 212—				
Steer H (period 2)	349	6,671	3,246	242
Steer H (period 4)	349	5,408	2,942	252
Steer H (period 6)	329	3,155	2,377	
Steer H (periods 2–6)				248
MAIZE STOVER:				
Experiment 210—				
Steer D (period 1)	345	4,335	2,919	550
Steer D (period 2)	331	3,548	2,486	−134
Steer D (period 3)	316	2,563	2,617	
Steer D (periods 1–3)				170
MAIZE MEAL ADDED TO CLOVER HAY:				
Experiment 179—				
Steer I (period 2)	520	[a] 3,144	3,679	1,300
Steer I (period 3)	514	3,898	4,659	598
Steer I (period 4)	532	6,637	6,296	
Steer I (periods 4–2)				748
WHEAT BRAN ADDED TO TIMOTHY HAY:				
Experiment 190—				
Steer A (period 3)	269	[a] 2,001	2,206	586
Steer A (period 1)	271	3,348	2,995	−288
Steer A (period 2)	279	4,579	2,639	
Steer A (periods 2–3)				168
Steer B (period 3)	194	[a] 1,774	1,774	−32
Steer B (period 1)	199	2,803	1,741	540
Steer B (period 2)	197	3,582	2,135	
Steer B (periods 2–3)				200
GRAIN MIXTURE NO. 1 ADDED TO TIMOTHY HAY:				
Experiment 200—				
Steer A (periods 3)	399	[a] 2,647	2,052	374
Steer A (period 1)	404	4,400	2,708	765
Steer A (period 2)	421	6,804	4,547	
Steer A (periods 2–3)				600
Steer B (period 3)	296	[a] 2,470	2,233	565
Steer B (period 1)	298	3,628	2,887	736
Steer B (period 2)	310	4,583	3,591	
Steer B (periods 2–3)				642

[a] Basal ration of coarse fodder only.

TABLE VII.—*Increments of heat production of cattle in standing*—Continued

Feeding stuff and experiment.	Average live weight.	Dry matter eaten per head.	Increment of heat production per 24 hours.	
			Per head.	Per kilogram of increment of dry matter eaten.
GRAIN MIXTURE NO. 1 ADDED TO TIMOTHY HAY—Continued.				
Experiment 207—	*Kilograms.*	*Grams.*	*Calories.*	*Calories.*
Steer A (period 3)	507	[a] 2,974	2,293	210
Steer A (period 1)	499	4,931	2,704	892
Steer A (period 2)	519	7,708	5,182	
Steer A (periods 2–3)				610
Steer B (period 3)	374	[a] 2,798	2,467	513
Steer B (period 1)	373	4,159	3,165	1,158
Steer B (period 2)	386	5,451	4,659	
Steer B (periods 2–3)				828
ALFALFA HAY AND GRAIN MIXTURE NO. 2:				
Experiment 208—				
Steer E (period 1)	210	3,208	1,944	180
Steer E (period 2)	206	1,753	1,682	−60
Steer E (period 3)	197	1,151	1,718	
Steer E (periods 1–3)				110
Steer C (period 2)	269	2,184	2,083	−110
Steer C (period 3)	259	1,539	2,154	
Experiment 209—				
Steer F (period 1)	301	4,502	2,442	348
Steer F (period 2)	293	2,756	1,834	−88
Steer F (period 3)	283	1,648	2,029	
Steer F (periods 1–3)				144
MIXED HAY AND MAIZE MEAL:				
Experiment 211—				
Steer G (period 2)	358	2,332	3,158	572
Steer G (period 3)	398	7,027	5,840	
MIXED HAY AND HOMINY CHOP:				
Experiment 211—				
Steer D (period 2)	432	3,511	3,533	86
Steer D (period 3)	470	7,859	3,905	

[a] Basal ration of coarse fodder only.

A simple inspection of Table VII suffices to show that the increment of heat production in standing can not be due to any large extent to the muscular work of supporting the body, since, in the light of Zuntz and Hagemann's (52) experiments on the horse, it must be assumed that this would be at least approximately proportional to the weight of the animal, while, in fact, in a large majority of cases the difference between the periods is very much greater than the corresponding difference in live weight. Even on the extreme assumption that in the periods on minimum rations the heat increment in standing was due exclusively to the increased muscular effort, the differences in live weight do not even remotely account for the greater increments in the other periods.

RELATION TO AMOUNT OF FEED

In spite of the considerable variations in the individual results and of some negative values, it appears clear, both from Table VII and from figure 1, especially if the comparisons be made between the smallest and the greatest rations, that the effect of the feed in increasing the metabolism tended to be distinctly greater during standing than during lying, and that, on the whole, the differences tended to be greater with concentrates or with mixed rations than with coarse feeds. In other words, the difference between the metabolism of the animal when standing and when lying was relatively greater on the heavier than on the lighter rations.

This difference can hardly be ascribed to a greater direct stimulus of cell metabolism by the products of digestion. One explanation for it might be sought in the fact that the feed was consumed by the animal while standing. Experiments by Paechtner (39) and by Dahm (17) on cattle and by Ustjanzew (46) on sheep, in which the respiratory exchange was determined in short periods, showed that the mastication of 1 kg. of hay increased the metabolism of the animal by approximately 60 Calories. In our experiments this would be equivalent to the production during perhaps half an hour after feeding—i. e., at 6 a. m. and 6 p. m.—of from 12 to as much as 200 Calories of heat, or twice this amount in 24 hours, which amounts would be added to the standing metabolism. On the other hand, however, according to the same experimenters, the rumination of the feed would increase the heat production by, roughly, two-thirds as much, and this would constitute to a considerable extent an addition to the metabolism of the animal when lying, thus partially but not wholly compensating for the addition to the metabolism when standing consequent on mastication.

It would appear, then, that the mastication of the heavier rations would tend to increase the ratio of heat production of the animal when standing to that when lying. The heat *elimination*, however, in our experiments showed no distinct evidence of such an increase. The rate of heat emission per minute while the feed was being eaten showed infrequently a slight rise, which was seldom sharp and which was far less than would correspond to the presumable increase in the gaseous exchange. In many instances no effect upon the heat elimination was observed, but rather frequently there was a distinct *fall*. Sometimes, although not in the majority of cases, a rise was observed after the animal had finished eating. The animal was watered after the 6 a. m. feeding. Sometimes no perceptible change in the rate of heat emission resulted, but not infrequently a fall was observed, which was occasionally considerable. It can hardly be doubted that there must have been an increased production of heat during mastication, but apparently this heat was not given off promptly. Part of it at least, it may be conjectured, was applied to warm

the ingesta, especially the water, being eliminated only gradually during the succeeding 12 hours and in part during the periods of lying as well as of standing. It does not seem probable, therefore, that the heat produced in the mastication of the feed was an important factor in causing the differences between the heat elimination of the animals when standing or lying which were observed in these experiments.

Another plausible suggestion seems to be a tendency of the animals to greater restlessness and muscular activity in the standing position when consuming the heavier rations. We are not able to submit direct records to prove this, but indirect evidence is afforded by the fact that the animals as a rule (31 cases out of 40) changed from the lying to the standing position, and vice versa, more frequently on the heavier rations. No such distinct effect was noticeable on the percentage of time spent standing, it being greater in 22 cases and less in 19 on the heavier as compared with the lighter rations and showing a considerable degree of constancy in the individual animal.

INDIVIDUAL DIFFERENCES

In those cases where comparisons between different animals are possible the average results, especially those obtained by comparing the maximum and minimum rations, seem to indicate the existence of distinct individual differences between different animals in this respect.

The most noticeable case of this sort is that of animals A and B, in experiments 190, 200, and 207, steer A being a typical beef animal, while B, although of mixed blood (scrub), was quite distinctly of the dairy type and of a more nervous temperament. In five cases out of six the heat increment due to standing was greater with steer B than with steer A, the average of all the results being 39 per cent higher for the former, as the following summary shows:

Heat increments in Calories per kilogram of dry matter due to standing

Feed.	Steer A.	Steer B.
Timothy hay	−88	148
	442	346
	364	628
Timothy hay and wheat bran	168	200
Timothy hay and grain mixture No. 1	600	642
	610	828
Average	335	465

A similar instance is afforded in experiments 208 and 209 by the animals C, E, and F. Steer C had received almost from birth as heavy feeding as practicable, while E and F had received the same feeds in quantities sufficient to insure normal growth but not to cause any material fattening. The heat increments per kilogram of dry matter of feed were as follows:

Heat increments in Calories per kilogram of dry matter due to standing

Feed.	Steer C.	Steer E.	Steer F.
Alfalfa hay	118	230	304
Alfalfa hay and grain mixture No. 2	—110	110	144
Average	4	170	224

INFLUENCE OF BULK OF RATION

It seems worth while to call attention also to three cases which suggest that the bulk of the ration may be a factor in determining the difference between the metabolism of the animals when standing and lying. In period 3 of experiments 208 and 209 steers C, E, and F received a light ration, two-thirds of which consisted of grain—i. e., a ration of small bulk—and on this light ration they showed a distinctly greater increment of metabolism during standing than upon one considerably heavier. It seems at least a plausible suggestion that the deficient bulk may have caused a greater degree of restlessness in period 3 and consequently an increased metabolism.

CORRECTION TO UNIFORM STANDING

Since standing or lying exerts such a marked influence on the heat production of animals, it is evident that a correction for this influence must be made before the results of the heat determinations can be regarded as comparable, since, notwithstanding the uniformity of external conditions striven after, the proportion of time spent standing or lying, respectively, varied more or less. As already pointed out this does not seem to have been related to the quantity of feed consumed, the difference having been practically as often in one direction as in the other. The stimulating effect, if such there were, seems to have expressed itself in more frequent changes of posture and a greater intensity of metabolism while standing rather than in more prolonged standing. The percentage of time spent standing appears to be largely a matter of individuality, whatever that convenient term may really signify. If we compare the results in this respect in the several periods, irrespective of the amount and kind of feed, it appears that, with a few exceptions, they show, on the whole, a rather marked degree of uniformity in the individual animal. This is especially true if experiment 190 be excepted, in which the animals were only about 12 months old. The data are contained in Table VIII, the averages in each case being computed, excepting the bracketed numbers.

TABLE VIII.—*Percentage of time spent standing*

Animal and experiment No.	Period No.	Percentage of time spent standing.
Steer I:		
174	A	57
	B	68
	C	57
	D	60
179	1	61
	2	63
	3	56
	4	[76]
186	1a	[87]
	2a	68
	3a	62
Average		65
186	1b	100
	2b	81
	3b	73
Average		85
Steer A:		
190	1	79
	2	79
	3	75
	4	81
Average		79
200	1	52
	2	58
	3	57
	4	51
207	1	50
	2	64
	3	46
	4	49
Average		53
Steer B:		
190	1	65
	2	46
	3	58
	4	28
200	1	47
	2	45
	3	57
	4	33
207	1	48
	2	46
	3	43
	4	39
Average		46
Steer C:		
208	2	[60]
	3	42
	4	47
	5	41
	6	42
Average		43
Steer D:		
208	1	32
	2	30
210	1	[53]
	2	38
	3	36
211	1	30
	2	40
	3	24
	4	36
	5	40
Average		34
Steer E:		
208	1	30
	2	27
	3	39
	4	40
	5	46
	6	[56]
Average		36
Steer F:		
209	1	27
	2	33
	3	32
	4	34
	5	33
	6	46
Average		34
Steer G:		
211	1	40
	2	42
	3	[28]
	4	35
	5	30
Average		37
Steer H:		
212	1	42
	2	40
	3	[29]
	4	38
	5	34
	6	37
Average		38

In view of this general uniformity, only a comparatively small correction would be necessary in most instances to make the results on an individual animal comparable as regards standing and lying. When, however, it is desired to compare the results obtained with different individuals so as to get a general average, it is clear that the animal which tends to stand most is at a disadvantage and will show a lower net energy value for the same ration not because his feed is any poorer but because the animal is a less efficient converter and that the greater the stimulus to metabolism exerted by standing the greater will this difference become. It is necessary, therefore, to correct the results upon heat production as well as possible to a uniform proportion of standing and lying, so as to render the results applicable to an (assumed) average animal. According to Table VIII the average percentage of time spent standing was 43. In correcting the results, however, we have used 50 per cent as the standard for convenience in calculation—that is, we have taken as the corrected heat production the average of that standing and lying, computed as shown on page 454 for the entire 48 hours.[1]

OTHER FORMS OF MUSCULAR ACTIVITY

According to our interpretation of our results, the material increase in the heat production of an animal when standing as compared with that when lying is simply an instance of the well-known influence of muscular exertion upon metabolism. Recent investigators, notably Schlossmann and Murschhauser (42, 43) and Benedict and Talbot (14, 15) in experiments upon infants, and Benedict and Homans (12, 13) in similar trials with dogs, have emphasized the disturbing influence of this factor upon comparisons of different periods. Benedict and his associates, in particular, have devised ingenious methods for determining the degree of muscular activity of a subject and have insisted that only periods of minimum activity can be safely compared.

We have not yet had the courage to attempt to apply to an animal weighing 1,000 or 1,200 pounds methods like those which have been used so successfully for infants and small dogs, either for the indication of minor movements or for the determination of the pulse rate. It seems likely that the latter, in particular, might be of considerable aid in the interpretation of the results if it should prove possible to devise a form of apparatus which would not be injured by the movements of a heavy animal.

[1] This method of correcting the results assumes that the relative intensity of the metabolism of the animal when standing or lying is not affected by the proportion of time spent standing. It might be imagined, however, that in a comparatively long period of standing the original stimulus to incidental muscular movements might gradually fade out, so that the average difference in the rate of metabolism in the two positions would be less for long than for short periods. In this case our method of correcting the results would be more or less erroneous. This possibility can be tested to a certain extent by comparing with each other the two single days of each period. Out of the 64 possible comparisons, that one of the two days in which the lesser percentage of time was spent standing showed a greater increment of standing over lying in 26 cases and a less increment in 37 cases. On the average of the 64 days of maximum standing the percentage of time spent standing was 51.9 and on the 64 days of minimum standing 43.5, while the corresponding average percentage increases in metabolism during standing were, respectively, 41.98 and 41.67. It does not appear, therefore, that within the range of these experiments the ratio of the metabolism when standing to that when lying was materially affected by the proportion of time spent standing.

In considering, however, the weight to be attached to the influence of variations in the muscular activity of the animal, and the degree of refinement necessary in experimental methods, it is important not to forget the main purpose of the experiments. This purpose was, as already explained, substantially an economic one—viz, to determine how much of the energy supplied in metabolizable form by the feed is, under ordinary conditions, dissipated through the heat production caused directly or indirectly by the ingestion of the feed and what proportion of it remains available for the physiological uses of the body. From this point of view it is immaterial whether the increased heat production is caused by "work of digestion" in the narrower sense, by the stimulating effect of the resorbed products of digestion upon the cell metabolism shown by the investigations of Lusk (31, 32, 33, 34, 35, 36, 49), or indirectly by giving rise to increased activity of the voluntary muscles. While it is of interest and value to learn as much as possible of the relative importance of these factors, nevertheless they are "all in the day's work," and from the economic viewpoint their aggregate constitutes the increased energy expenditure consequent upon feed consumption. Even if it were practicable to base comparisons upon periods of minimum activity the results, however interesting physiologically, would include only a part of the effects which the feed actually exerts upon the metabolism. If the feed causes greater restlessness in the animal while standing or causes it to get up and to lie down more frequently, this gives rise, under the conditions of practice, to just as real losses of energy as does the increase of the general cell metabolism when in the lying position and from the economic point of view must be taken into account. What is needed is a comparison of periods of *average* rather than of minimum muscular activity and the correction to 12 hours of standing aims to reduce conditions to such an average (assumed) as regards this very important factor.

Of course, however, the possibility of variations in other forms of muscular activity, arising from differences in external conditions other than the feed, has to be reckoned with. Naturally the endeavor has been to make those conditions as nearly uniform as possible. The feeding was identical from day to day during the three weeks of each period and was given at the same hours. The surroundings during the days spent outside the calorimeter were uniform, and the animals were handled by the same attendants.

During the days in the calorimeter even greater uniformity of conditions existed. The temperature varied only a few hundredths of a degree, the triple walls of the apparatus practically shut off all external sounds except the slight monotonous click of the meter pump, while in the comparatively dim interior the change outside from daylight to artificial light could not have been very noticeable. Visitors were not admitted during the runs. As already stated, all the animals were docile

and accustomed to being handled, to the wearing of the apparatus for the collection of excreta, and to the presence of the observers.

Some idea as to the extent to which these precautions were successful may be formed from a comparison of the quantities of heat produced after correction to 12 hours standing on the two successive days of each 48-hour calorimeter run. The first half of Table IX shows the corrected heat production on the first and second days of each run and likewise the mean, computed in the manner illustrated on page 454 for the entire 48 hours.[a]

TABLE IX.—*Heat production per day and per head corrected to 12 hours standing*

Feeding stuff and experiment No.	Animal No.	Period No.	Corrected heat production.			Analysis of heat production.			
			First day.	Second day.	48-hour mean.	Standing 12 hours.	Rising and lying down.	CH_4 fermentation.	Remainder.
Timothy hay:			*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
174	I	A	8,788	9,316	9,049	1,112	17	448	7,472
	I	B	9,899	9,565	9,769	1,450	18	570	7,731
	I	C	10,309	10,723	10,562	1,440	17	626	8,479
	I	D	11,013	11,130	11,149	1,437	27	864	8,821
190	A	3	5,709	5,532	5,636	1,091	12	282	4,251
	A	4	7,535	6,545	6,709	1,023	15	481	5,190
	B	3	4,985	4,904	5,081	875	12	276	3,918
	B	4	6,095	5,886	5,852	937	12	361	4,542
200	A	3	7,186	7,275	7,245	1,001	25	[b] 378	5,841
	A	4	8,057	8,205	8,186	1,379	40	[b] 606	6,161
	B	3	6,773	6,707	6,778	1,093	24	[b] 367	5,294
	B	4	7,750	7,783	7,837	1,315	33	[b] 540	5,949
207	A	3	7,713	7,812	7,791	1,107	40	498	6,146
	A	4	9,267	9,649	9,523	1,438	59	794	7,232
	B	3	7,996	8,070	8,064	1,190	43	481	6,350
	B	4	9,621	9,637	9,812	1,758	51	747	7,256
Red clover hay:									
179	I	1	10,939	10,910	10,926	2,354	43	626	7,903
	I	2	9,327	9,944	9,621	1,812	28	464	7,317
186	I	1a	9,844	9,586	9,627	1,604	20	413	7,590
	I	3a	9,835	10,618	10,176	1,958	51	464	7,703
	I	2a	10,335	10,945	10,574	2,183	36	555	7,800
	I	3b	10,178	10,197	10,206	1,492	48	464	8,202
	I	2b	10,274	10,976	10,501	1,688	38	555	8,220
Mixed hay:									
211	D	1	11,775	12,995	12,359	1,679	79	805	9,796
	D	4	9,481	9,680	9,625	1,498	76	527	7,524
	D	5	8,291	8,259	8,258	1,497	57	282	6,422
211	G	1	12,211	11,971	12,098	1,802	80	830	9,386
	G	4	9,843	9,242	9,568	1,958	75	457	7,078
	G	5	7,811	7,178	7,477	1,382	62	261	5,772

[a] The 48-hour means differ from the means of the 24-hour periods for two reasons: First, the percentage increase of the metabolism when standing over that when lying varied in the two days, as did also the percentage of the total heat carried off as latent heat of water vapor. On account especially of the latter difference, the mean of the two days taken singly differs from that computed from the average heat production per minute standing and lying for the whole 48 hours. Second, in experiments 190, 200, and 207 the corrected results for the 48 hours are computed from selected portions of the runs in the manner described in an earlier publication (10, p. 43), and therefore differ from the mean of the results for the single days.

[b] Methane computed from digested carbohydrates.

TABLE IX.—*Heat production per day and per head corrected to 12 hours standing*—Con.

Feeding stuff and experiment No.	Animal No.	Period No.	Corrected heat production.			Analysis of heat production.			
			First day.	Second day.	48-hour mean.	Standing 12 hours.	Rising and lying down.	CH_4 fermentation.	Remainder.
			Cals.	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
Alfalfa hay:									
	D	1	5,385	5,442	5,411	1,000	39	256	4,116
	D	2	4,243	4,290	4,266	612	45	161	3,448
	E	4	7,931	7,823	7,879	1,014	58	457	6,350
	E	5	5,759	5,764	5,764	796	45	302	4,621
208	E	6	4,529	4,590	4,566	732	24	180	3,630
	C	4	8,965	9,100	9,052	1,297	48	470	7,237
	C	5	7,340	7,391	7,357	1,292	38	380	5,647
	C	6	6,136	6,122	6,124	1,156	34	271	4,663
	F	4	11,228	11,432	11,474	1,400	86	697	9,291
209	F	5	.....	8,031	8,007	948	43	429	6,587
	F	6	6,819	6,794	6,781	830	54	285	5,612
	H	1	11,424	11,186	11,272	1,522	62	837	8,851
212	H	3	10,189	10,616	10,388	1,549	63	685	8,091
	H	5	7,836	7,582	7,754	1,196	45	415	6,098
Alfalfa meal:									
	H	2	11,025	11,505	11,252	1,570	53	760	8,869
212	H	4	9,889	10,270	10,066	1,425	46	638	7,957
	H	6	7,027	7,132	7,069	1,146	42	419	5,462
Maize stover:									
	D	1	9,550	9,203	9,363	1,428	31	495	7,409
210	D	2	8,605	8,404	8,495	1,199	44	405	6,847
	D	3	7,527	7,429	7,476	1,250	59	308	5,859
Maize meal added to clover hay:									
	I	[a] 2	9,327	9,944	9,621	1,812	28	464	7,317
179	I	3	10,483	9,876	10,198	2,284	45	630	7,239
	I	4	12,723	13,354	12,947	3,124	21	1,110	8,689
Wheat bran added to timothy hay:									
	A	[a] 3	5,709	5,532	5,636	1,091	12	282	4,251
	A	1	7,819	7,328	7,739	1,486	12	503	5,738
	A	2	.....	8,400	8,385	1,307	13	643	6,422
190	B	[a] 3	4,985	4,904	5,081	875	12	276	3,918
	B	1	6,493	6,299	6,363	865	6	400	5,092
	B	2	6,814	7,117	7,409	1,057	11	537	5,804
Grain mixture No. 1 added to timothy hay:									
	A	[a] 3	7,186	7,275	7,245	1,001	25	[a] 378	5,841
	A	1	8,955	9,236	9,274	1,322	32	[a] 679	7,241
200	A	2	12,140	12,667	12,514	2,226	48	[a] 1,043	9,197
	B	[a] 3	6,773	6,707	6,778	1,093	24	[a] 367	5,294
	B	1	8,916	8,952	9,016	1,427	17	[a] 556	7,016
	B	2	9,810	9,710	9,909	1,771	25	[a] 684	7,429
	A	[a] 3	7,713	7,812	7,791	1,107	40	498	6,146
	A	1	9,786	10,181	10,164	1,294	58	841	7,971
	A	2	13,534	12,738	13,375	2,518	73	1,222	9,562
207	B	[a] 3	7,996	8,070	8,064	1,190	43	481	6,350
	B	1	9,434	9,568	9,600	1,558	24	733	7,285
	B	2	11,658	11,640	11,720	2,285	45	954	8,436

[a] Basal ration of coarse fodder only.

TABLE IX.—*Heat production per day and per head corrected to 12 hours standing*—Con.

Feeding stuff and experiment No.	Animal No.	Period No.	Corrected heat production.			Analysis of heat production.			
			First day.	Second day.	48-hour mean.	Standing 12 hours.	Rising and lying down.	CH_4 fermentation.	Remainder.
Alfalfa hay and grain mixture No. 2:			*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
208	E	1	7,434	7,532	7,483	916	56	464	6,047
	E	2	5,940	5,911	5,924	788	53	303	4,780
	E	3	5,032	5,138	5,084	824	35	165	4,060
	C	2	6,802	6,927	6,858	1,011	31	388	5,428
	C	3	6,096	6,149	6,123	1,043	34	284	4,762
209	F	1	9,679	10,099	9,888	1,132	89	562	8,105
	F	2	7,645	7,809	7,115	844	73	382	5,816
	F	3	6,791	6,659	6,734	948	68	253	5,465
Mixed hay and maize meal:									
211	G	2	8,678	8,362	8,470	1,505	74	470	6,421
	G	3	14,510	14,467	14,561	2,852	68	1,126	10,515
Mixed hay and hominy chop:									
211	D	2	9,782	10,126	9,947	1,695	71	643	7,538
	D	3	14,877	15,040	14,936	1,850	102	1,216	11,768

As already stated, we estimate the experimental error in the determination of the heat emitted by the animal to be approximately 1 per cent. In the 73 cases in which a comparison of the two days can be made, 40 show a deviation from the mean of the two 24-hour results of less than 1.1 per cent—i. e., the results for the two days practically agree within the limits of experimental error. Of the 53 experiments made since 1905—i. e., experiments 200 to 212—which, in our judgment, are, on the whole, more accurate than the earlier ones, 36 fall within this limit or error. On the other hand, however, deviations as great as 2 per cent are not uncommon, while occasionally they rise to as much as 5 per cent or even 7 per cent. The mean of the percentage deviations is for the entire series 1.45 per cent and for experiments 200 to 212, inclusive, 1.13 per cent. Moreover, the deviations of the single days from the mean are, on the whole, fully as great after reduction to 12 hours standing as before. It is clear, therefore, that despite the apparent uniformity of experimental conditions the metabolism of the animals was affected by influences other than the feed or the proportion of time spent standing.

Mr. H. H. Mitchell, of the Illinois Experiment Station, has had the kindness to submit these data to mathematical study and finds that they present clear evidence of the existence of individual differences between the animals as regards the agreement between the two days. He writes as follows:

It seems very evident to me both from inspection of the data and from statistical calculations that the percentage deviation of duplicate determinations of the heat

production of animals is variable, depending undoubtedly upon the particular animal under investigation and possibly, also, upon the nature of the ration. Thus the average percentage deviations for the four animals for which 10 or more observations are recorded, with their probable errors, are:

Animal I	2.10±0.23
Animal A	[a] 2.25±0.35
Animal A	[b] 1.77±0.18
Animal B	0.75±0.13
Animal D	1.32±0.28

While, according to these figures, animals I and A can not be differentiated from each other, they are both clearly differentiated from animal B, the differences, with their probable errors, being 1.35±0.26 between animals I and B and 1.02±0.22 between animals A and B. The difference between animals I and D was 0.78±0.36, the significance of which may be questioned. Using another method of comparison, it may be shown that the odds are 124 to 1 that animals C and H are definitely distinct as regards the percentage deviation under discussion. I should not hesitate to conclude, therefore, that this percentage deviation is affected by the individuality of the experimental animals. Furthermore, there is a slight suggestion, especially in the data of animals A and B, that the nature of the ration may affect the percentage deviation of your determinations.

It is of interest to note in this connection that the results of Kellner's respiration experiments (uncorrected for standing or lying) likewise show variations of much the same order of magnitude between individual (not consecutive) days. When, therefore, comparisons are based upon the average results for 48 hours, it is impossible to assert that these results represent, as they should, periods of average muscular activity, although it would appear that the error thus introduced is usually not large. In Kellner's experiments it is still further reduced by the fact that in most cases the results of four or five single runs are averaged.

ANALYSIS OF HEAT PRODUCTION

In Table VII were shown the increments of heat production per 24 hours in standing animals as compared with those lying. It is evident that of the total corrected heat production recorded in Table IX an amount equal to one-half of the corresponding increment shown by Table VII is to be regarded as the effect of the 12 hours' standing, while the remainder represents the metabolism of the animal per 24 hours lying. On the basis of Zuntz's recent results it is possible to carry this analysis of the heat production a little farther, at least approximately. The expenditure of energy caused by standing obviously includes that required for the muscular effort of rising and lying down. Von der Heide, Klein, and Zuntz (20, p. 823) estimate this on the basis of experiments by Klein at 9.7 Calories per 550 kg. of live weight for once rising and lying down again. The same investigators (20, p. 795) compute from Markoff's experiments (37, 38) that the methane fermentation in cattle gives rise to the evolution of 4.374 Calories of heat per cubic centimeter of methane, equivalent to 6.07 Calories per gram. While both the foregoing figures are confessedly but approximations, nevertheless they permit a partial analysis of the heat production with

[a] Including questionable observation. [b] Not including questionable observation.

the results shown in the second part of Table IX. The "remainder" shown in the last column includes the so-called basal or fasting metabolism, together with the effect of the feed in increasing the muscular activity of the organs of digestion and of the voluntary muscles in the lying position, as well as in directly stimulating the cell metabolism. No sufficient data are available for further analysis of this "remainder."

PROPORTION OF FEED ENERGY EXPENDED IN HEAT PRODUCTION

METHOD OF DETERMINATION

The total metabolism of an animal upon any particular ration, as illustrated by the figures of Table IX, is made up of numerous factors, and a single experiment affords no means of determining the proportion due to the consumption of feed. This can be determined only by a comparison of two periods, otherwise identical, in which different quantities of the same feed are consumed, the additional heat production on the heavier ration constituting the measure of the additional energy expended. With carnivora and with man the comparison may be made with the fasting state—i. e., the amount of feed in one of the periods may be zero. With cattle this is impracticable for obvious reasons, and it is necessary to make the comparison with a period upon a so-called basal ration. Kühn and Kellner added the feeding stuffs to be tested to a mixed basal ration that was more than sufficient for maintenance. In our earlier tests, up to experiment 207, inclusive, the same general plan was followed, except that the basal ration consisted of coarse feeds only and was in most cases below the maintenance requirement. In the later experiments the method was modified by feeding different quantities of the same feed or mixture of feeds both above and below the maintenance requirement. The method of comparison for a ration consisting of a single feeding stuff is very simple. Thus, in experiment 207 the following results were obtained on timothy hay with steer A in periods 3 and 4. The same method of comparison may obviously be applied also to different amounts of a mixed ration of grain and hay.

Computation of energy expenditure by steer A per kilogram of timothy hay eaten

Item.	Quantity of dry matter eaten.	Total heat production.	Distribution of heat production.			
			Standing.	Rising and lying down.	Fermentation.	Remainder.
	Gm.	*Calories.*	*Calories.*	*Calories.*	*Calories.*	*Calories.*
Period 4	4,892	9,523	1,438	59	794	7,232
Period 3	2,974	7,791	1,107	40	498	6,146
Difference	1,918	1,732	331	19	296	1,086
Difference per kilogram of dry matter	.....	903	173	9	154	567

The comparison of these two periods shows that each additional kilogram of dry matter consumed increased the total heat production by 903 Calories, 173 of which represent energy expended in standing, 9 that expended in rising and lying down, and 154 the additional heat due to the methane fermentation, while the remainder, 567 Calories, represents the increased mechanical work of digestion plus any stimulus which the digested nutrients exerted upon the cell metabolism. Obviously the calculation by difference eliminates the basal metabolism.

For concentrated feeds, which can not be fed alone, two methods have been used, as already noted. In the earlier experiments, the concentrate was added to the basal ration of coarse fodder. Thus, in period 2 of the experiment just used as an illustration a mixture of grains (grain mixture No. 2) was added to the basal ration of period 3 with the following results:

Computation of energy expenditure by steer A per kilogram of grain eaten

Item.	Quantity of dry matter eaten.		Total heat production.	Distribution of heat production.			
	Hay.	Grain.		Standing.	Rising and lying down.	Fermentation.	Remainder.
	Gm.	*Gm.*	*Calories.*	*Calories.*	*Calories.*	*Calories.*	*Calories.*
Period 2	2,949	4,759	13,375	2,518	73	1,222	9,562
Period 3	2,974	.....	7,791	1,107	40	498	6,146
Difference	−25	4,759	5,584	1,411	33	724	3,416
Difference per kilogram of dry matter	.....	.....	1,179	298	7	153	721

Each kilogram of dry matter of the grain increased the heat production by 1,179 Calories, which can be subdivided as before in the proportions shown.[1] The greater expenditure of energy per kilogram in the case of grain as compared with hay is seen to be due in part to a greater increase of the metabolism of the animal when standing and in part either to increased mechanical work in digestion or more likely to a greater stimulus of the cell metabolism.

In later experiments (Nos. 208 to 212, inclusive), in place of adding grain to a ration of coarse fodder, the animals received varying quantities of a uniform mixture of coarse fodder and grain, the energy expenditure caused by the total ration being determined substantially in the manner already illustrated. The portion of the increase due to the grain alone was computed by subtracting from the total increase that due to the hay as determined in two or more separate periods on exclusive hay rations.[2] The method may be illustrated by the results obtained with steer E in periods 1 and 3 of experiment 208.

[1] Logically the results of the comparison should be corrected for the slight difference (25 gm.) in the amount of dry matter of hay consumed. As a matter of fact, however, this correction is insignificant in all the experiments, amounting in the present instance to about 1 Calorie.

[2] When more than two periods of hay feeding were used the increased heat production per kilogram of hay was computed by comparing the periods on the heaviest and the lightest rations.

Computation of energy expenditure of steer E per kilogram of grain eaten

Item.	Quantity of dry matter eaten.		Total heat production.	Distribution of heat production.			
	Hay.	Grain.		Standing.	Rising and lying down.	Fermentation.	Remainder.
	Gm.	*Gm.*	*Calories.*	*Calories.*	*Calories.*	*Calories.*	*Calories.*
Period 1	1,086	2,122	7,483	916	56	464	6,047
Period 3	387	764	5,084	824	35	165	4,060
Difference	699	1,358	2,399	92	21	299	1,987
Difference due to 699 gm. of hay [a]	.....	.....	840	71	9	70	690
Difference due to 1,358 gm. of grain	.....	.....	1,559	21	12	229	1,297
Difference per kilogram of grain	.....	.....	1,148	15	9	169	955

[a] Computed from a comparison of periods 4 and 6.

DIFFERENCES IN LIVE WEIGHT

As the figures of Table VII show, the live weight of the animals varied more or less in the different periods. To what extent do these variations affect the conclusions drawn from comparisons like those just illustrated?

Two effects other than those due directly to the amount of feed might be anticipated from an increase in the live weight: First, an increase in the basal metabolism due to a greater mass of tissue, and, second, an increase in the muscular work of supporting the body in the standing position. As regards the first of these, it is to be remarked that the experimental periods were short (three or, in a few cases, four weeks only) while the changes in the amount and kind of feed consumed were considerable. It seems altogether probable that the larger part of the variation in weight must be ascribed to "fill"—i. e., to variations in the contents of the digestive tract rather than to any considerable change in the make-up of the body proper—and that the actual basal metabolism was not greatly affected. As regards the effect upon the muscular work of standing, it has been already pointed out that this appears to be a relatively small factor in the total increase of heat elimination in standing. In view of these considerations, it is to be anticipated that a correction of the heat production in proportion to either the weight or the surface of the animals would materially exaggerate the effect upon the metabolism, and, on the whole, we have regarded it as safer to disregard the variations in live weight rather than to attempt a more or less conjectural correction.

HEAT INCREMENTS PER KILOGRAM OF DRY MATTER

The results of the comparisons between periods made by the methods just illustrated are contained in Table X. In those cases in which more than two periods upon the same ration can be compared, the total heat increments per kilogram of dry matter are recorded for each successive

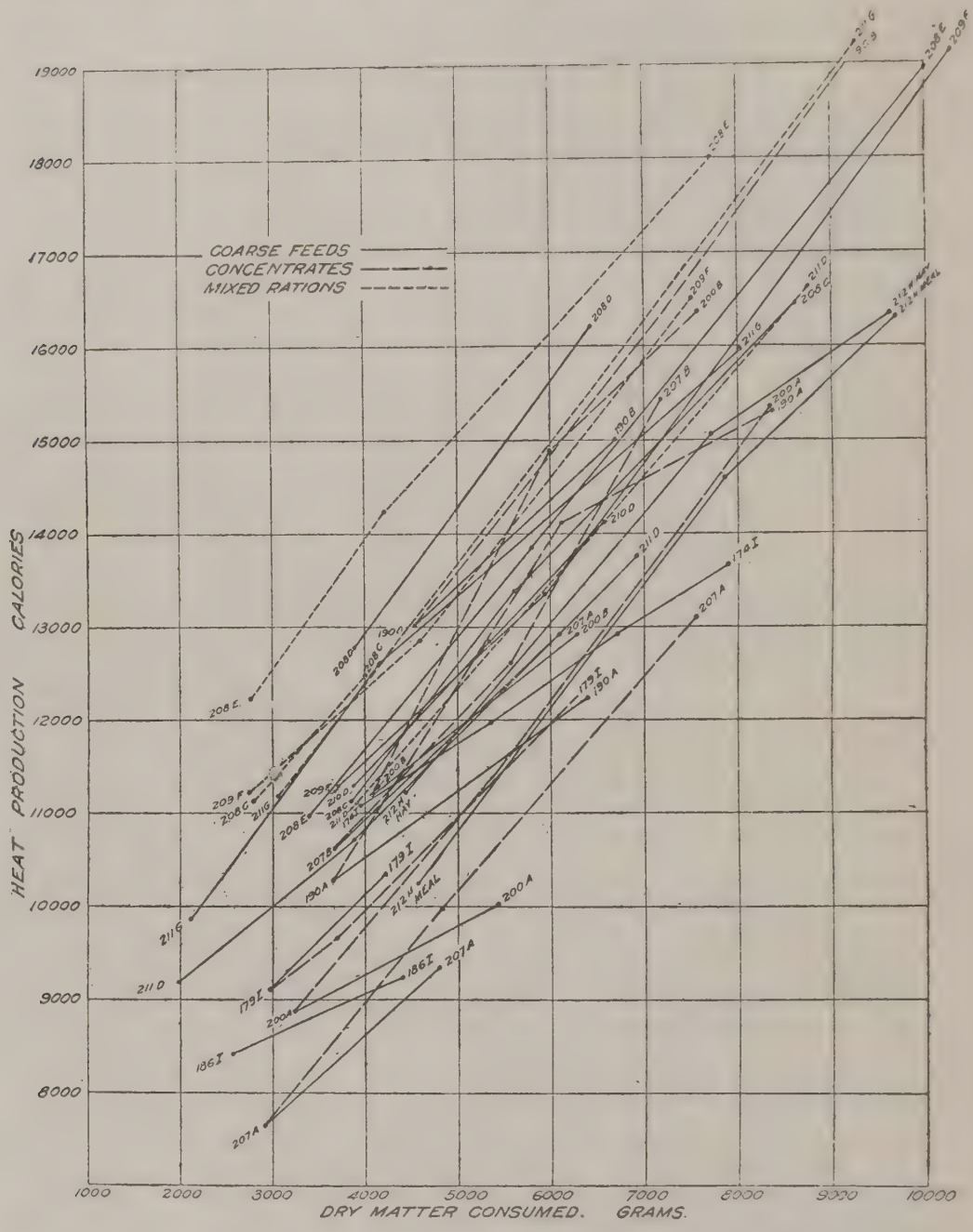

FIG. 2.—Graph showing the relation of heat production to dry matter consumed; computed per 500 kg. live weight.

pair of periods, beginning with the lighter ration. Thus, in the case of steer I in experiment 174 four different amounts of timothy hay [1] were eaten. Table X shows the total heat increments computed by comparing periods A with B; B with C; C with D; and finally A with D. The

[1] About 350 gm. of linseed meal in each period were also fed.

final comparison, between the smallest and greatest rations, is regarded as the average result, and on it is based the computation of the distribution of this energy between standing, rising, and lying down, fermentation, and the "remainder," as well as, in the case of mixed rations, the computation of the energy increment due to the hay. The same results, computed per 500 kg. of live weight, are also represented graphically in figure 2, the abscissæ representing the amount of dry matter consumed and the ordinates the corresponding corrected heat production, the heat production per kilogram of dry matter corresponding to the tangent of the angle between the graph and the horizontal axis.

TABLE X.—*Increments of heat production per kilogram of dry matter*

Feeding stuff and experiment No.	Animal No.	Successive amounts of feed.			Comparison of greatest and least amounts of feed.	Analysis of heat increments.			
						Standing 12 hours.	Rising and lying down.	CH_4 fermentation.	Remainder.
Timothy hay:		*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
174	I	634	716	612	656	102	3	130	421
190	A	.....	.....	.....	719	−46	2	133	630
	B	.....	.....	.....	922	74	0	102	746
200	A	.....	.....	.....	529	213	8	128	180
	B	.....	.....	.....	793	166	7	130	490
207	A	.....	.....	.....	903	173	9	154	567
	B	.....	.....	.....	954	310	4	145	495
Average		.....	.....	.....	782	141	5	132	504
Red clover hay:									
179	I	.....	.....	.....	992	412	11	123	446
186	I, series *a*	455	449	.....	453	277	8	68	100
Average		.....	.....	.....	723	344	10	96	273
186	I, series *b*	.....	.....	.....	333	220	−10	103	20
Mixed hay:									
211	D	799	1,010	.....	928	41	5	118	764
	G	1,357	860	.....	1,031	94	4	137	806
Average		1,078	935	.....	980	67	5	123	785
Alfalfa hay:									
208	D	.....	.....	.....	1,339	454	− 7	111	781
	E	1,204	1,200	.....	1,202	102	13	100	987
	C	1,260	1,030	.....	1,116	54	5	76	981
209	F	918	1,327	.....	1,189	145	8	104	932
212	H	1,161	671	.....	981	91	5	118	767
Average		1,147	1,051	.....	1,165	169	5	102	889

TABLE X.—*Increments of heat production per kilogram of dry matter*—Continued

Feeding stuff and experiment No.	Animal No.	Successive amounts of feed.			Comparison of greatest and least amounts of feed.	Analysis of heat increments.			
						Standing 12 hours.	Rising and lying down.	CH_4 fermentation.	Remainder.
		Cals.	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
Alfalfa meal:									
212	H	1,330	939	.....	1,190	121	3	97	969
Average of alfalfa hay and meal		1,184	1,028	.....	1,169	161	5	101	902
Maize stover:									
210	D	1,034	1,101	.....	1,065	101	−16	105	875
Alfalfa hay and grain mixture No. 2:									
208	E	1,395	1,072	.....	1,166	45	10	145	966
	C	.....	.....	.....	1,139	−50	− 5	161	1,033
209	F	886	1,244	.....	1,105	64	7	108	926
Average		1,146	1,160	.....	1,139	20	4	138	977
Alfalfa hay and grain mixture No. 2 (periods 1 and 2):									
208	E	.....	.....	.....	1,072	88	2	111	871
	C	.....	.....	.....	.....	....	....	....	.....
209	F	.....	.....	.....	1,248	169	9	103	971
Average		.....	.....	.....	1,160	127	5	107	921
Mixed hay and maize meal:									
211	G	.....	.....	.....	1,297	287	−1	140	871
Mixed hay and hominy chop:									
211	D	.....	.....	.....	1,147	36	7	132	972
Maize meal added to clover hay:									
179	I	765	1,004	.....	952	375	−1	185	393
Maize meal (computed):									
211	G	.....	.....	.....	1,434	386	−4	146	906
Hominy chop (computed):									
211	D	.....	.....	.....	1,365	30	9	146	1,180
Wheat bran added to timothy hay:									
190	A	1,561	525	.....	1,066	84	0	140	842
	B	1,246	1,343	.....	1,288	101	−1	144	1,044
Average		1,404	934	.....	1,177	93	−1	142	943

TABLE X.—*Increments of heat production per kilogram of dry matter*—Continued

Feeding stuff and experiment No.	Animal No.	Successive amounts of feed.			Comparison of greatest and least amounts of feed.	Analysis of heat increments.			
						Standing 12 hours.	Rising and lying down.	CH_4 fermentation.	Remainder.
		Cals.	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
Grain mixture No. 1 added to timothy hay:									
200	A	1,157	1,348	.....	1,267	294	6	160	807
	B	1,933	935	.....	1,482	321	0	150	1,011
207	A	1,213	1,156	.....	1,179	298	7	153	721
	B	1,129	1,640	.....	1,378	413	1	178	786
Average		1,358	1,270	.....	1,327	331	4	161	831
Grain mixture No. 2 (computed):									
208	E	1,494	1,004	.....	1,148	15	9	169	955
	C	.....	.....	.....	1,152	−99	−9	202	1,058
209	F	759	1,277	.....	1,077	27	7	110	933
Average		1,127	1,141	.....	1,125	−19	2	160	982
Grain mixture No. 2 (computed from periods 1 and 2):									
208	E	.....	.....	.....	1,004	80	−3	116	811
209	F	.....	.....	.....	1,277	175	9	103	990
Average		.....	.....	.....	1,141	128	3	110	900

CRITICAL TEMPERATURE

In order that comparisons like the foregoing shall be valid, the experiments must, of course, be made above the so-called "critical temperature" for the animal experimented with and for the minimum quantity of feed consumed, since below this temperature part of the heat produced is utilized to maintain the body temperature and thus to reduce the amount of heat liberated by the katabolism of body substance (2, p. 347–359, 407–410). Our experiments have been made at about 17° to 18° C., and we have not attempted to determine the critical temperature for cattle, but the fact clearly shown in figure 2 that the heat production per kilogram of feed consumed showed no tendency to increase as the rations were made heavier leads us to believe that even on the lightest rations the temperature was safely above the point at which the so-called "chemical" regulation of body temperature begins. Kellner's experiments were made at somewhat lower temperatures, mostly between 12° and 15° C., but on heavier rations.

DISCREPANCIES IN RESULTS

It is apparent from both Table X and figure 2 that the single results show a considerable range for the same or similar feeds not only with different animals but also, in some instances, between different periods with the same animal. For example, in experiment 209, on steer F with alfalfa hay an increase of the ration from 2,226 to 3,562 gm. of dry matter caused an increase in the (corrected) heat production at the rate of 963 Calories per kilogram, while a further increase to 6,174 gm. resulted in a relatively greater increase of the heat production—viz, 1,301 Calories per kilogram.

In the instance just cited one might be inclined to interpret the difference as an effect of the greater feed consumption. The next line in Table X, however, shows an even greater difference in the opposite direction, while it is evident from figure 2 that the data as a whole show about as many differences in one direction as the other and, as pointed out in the previous paragraph, fail to give any distinct evidence of a greater relative increase of heat production on heavy as compared with light feed or on supermaintenance as compared with submaintenance rations, the averages tending, if anything, to be a trifle lower on the heavier rations.

Unavoidable differences in the muscular activities of the animal, other than those connected with standing and lying, and in other conditions have also to be considered. As already pointed out, the existence of such differences, in spite of the uniformity of the controllable experimental conditions, is indicated by the occasionally considerable divergence of the heat production upon the two days of the calorimeter runs. It is not improbable, therefore, that they may be responsible, at least in part, for the observed discrepancies, so that it is obvious that the average results must be accepted with some reserve. On the other hand, however, it must be remembered that these are calculations by difference and that in such a calculation the experimental errors tend to accumulate in the final result. Obviously the greater we make the difference in the factor whose effect is to be determined, the less will be the relative error of the final result.[1] We believe, therefore, that the results obtained by a comparison of the extreme rations, as recorded in column 4 of Table X, are decidedly more trustworthy than those computed from the intermediate rations, and, notwithstanding the discrepancies just mentioned, are inclined to regard them as expressing the total effect of the feed in increasing the metabolism, when variations in the time of standing are eliminated, with a sufficient degree of accuracy to warrant general comparisons of the average results. These average results, both as to the total heat increment and its factors, are summarized in Table XI.

[1] Out of 13 cases in which the results appear abnormally high or low, there were 6 in which the difference in dry matter consumed was less than 1 kg., although there were 5 other cases in which, with a similar small difference in the dry matter consumed, apparently normal results were obtained.

TABLE XI.—*Average increments of heat production per kilogram of dry matter*

Feeding stuff.	Total increment.	Analysis of heat increment.			
		Standing 12 hours.	Rising and lying down.	CH_4 fermentation.	Remainder.
COARSE FODDERS:	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
Timothy hay	782	141	5	132	504
Red clover hay:					
Average	723	344	10	96	273
Experiment 179	992	412	11	123	446
Mixed hay	980	68	4	123	785
Alfalfa hay	1, 165	169	5	102	889
Alfalfa meal	1, 190	121	3	97	969
Average of alfalfa hay and meal	1, 169	161	5	101	902
Maize stover	1, 065	101	−16	105	875
MIXED RATIONS:					
Alfalfa hay and grain mixture No. 2					
Average of all	1, 139	20	4	138	977
Average of periods 1 and 2 only	1, 160	127	5	107	921
Mixed hay and maize meal	1, 297	287	− 1	140	871
Mixed hay and hominy chop	1, 147	36	7	132	972
CONCENTRATES:					
Maize meal added to clover hay	952	375	− 1	185	393
Maize meal computed from mixed ration	1, 434	386	− 4	146	906
Hominy chop computed from mixed ration	1, 365	30	9	146	1, 180
Wheat bran added to timothy hay	1, 177	93	1	142	943
Grain mixture No. 1 added to timothy hay	1, 327	331	4	161	831
Grain mixture No. 2 computed from mixed ration	1, 125	−19	2	160	982
The same from periods 1 and 2 only	1, 141	128	3	110	900

COMPARISON OF COARSE FEEDS AND CONCENTRATES

The average results recorded in Table XI for the total increase in metabolism resulting from the consumption of 1 kg. of dry matter of the several rations—i. e., for the so-called "work of digestion" in the widest sense—are far from being in accord with common conceptions. Unconsciously misled by an unfortunate terminology, we have been accustomed to think of the more coarse and woody feeds, like hay, straw, stover, etc., as requiring a greater expenditure of energy in their digestion and assimilation than the more concentrated and highly digestible grains, for example. It may be somewhat surprising, therefore, to note the relatively small differences found in this respect between different classes of feeding stuffs, as shown by the averages of Table XI and by figure 2. For example, the expenditure of energy caused by maize meal in experiment 179 was almost as great as that caused by

the clover hay with which it was fed, while in experiment 211 it was apparently distinctly greater than that due to the mixed hay consumed with it. Grain mixture No. 1 decidedly exceeded timothy hay in this respect, and grain mixture No. 2 was nearly equal to alfalfa hay.

As a matter of fact, however, these results are in general harmony with those of other investigators, particularly Kellner. The senior writer (2, p. 492) pointed out some 12 years ago that the total expenditure of energy consequent upon feed consumption, as computed from Kellner's published experiments, is strikingly uniform for the several materials experimented upon with the exception of wheat gluten, the average results computed per kilogram of dry matter being quite of the same order as those here reported, viz:

Average energy expenditure per kilogram of dry matter

	Calories.
Meadow hay	1,254
Oat straw	1,014
Wheat straw	1,138
Extracted straw	1,160
Starch:	
Kühn's experiments	1,508
Kellner's experiments—	
Moderate rations	1,248
Heavy rations	903
Peanut oil	[a] 1,727
Wheat gluten:	
Kühn's experiments	2,558
Kellner's experiments	2,096
Beet molasses	988

Kellner's later experiments (24, ed. 6, p. 160–168) have not yet been published in full, so that it is not possible to make an exact computation of the energy expenditure. In certain cases, however, the percentages of digestible nutrients are reported. If the corresponding amount of metabolizable energy be computed, using the factors given on page 453, and from this the amount of energy gained by the animal subtracted, the difference will represent approximately the energy spent in digestion, etc. The results of such computations are as follows:

Energy expenditure per kilogram of dry matter, computed from Kellner's experiments

Feeding stuff.	Digestible nutrients.	Computed metabolizable energy.	Gain in energy by animal.	Energy expended in feed consumption.
	Gm.	*Calories.*	*Calories.*	*Calories.*
Cottonseed meal	647	2,588	1,869	719
Peanut meal	672	2,688	1,798	890
Palm-nut meal	624	2,496	1,739	757
Linseed meal	690	2,760	1,828	932
Barley straw	464	1,624	747	877
Clover hay	498	1,743	811	932
"Grass hay"	528	1,848	803	1,045
Rowen	487	1,705	747	958

The approximate results thus computed for the coarse fodders are comparable in a general way with ours upon similar feeds, although

[a] One very high result was rejected.

somewhat lower than Kellner's direct results just cited. Those on the oil meals appear relatively lower than ours, although even then they are not much lower than those for the coarse feeds, but it may be questioned whether the estimates of the metabolizable energy of these feeds are not too low.

FACTORS OF INCREASED METABOLISM

Even the very approximate and partial analysis of the total heat production which is attempted in the second part of Tables IX, X, and XI serves to show that the degree of uniformity noted in the preceding paragraph, so far from being surprising, was rather to be expected. The notion of a greater expenditure of energy on coarse feeds is based on the idea that this expenditure is largely for mechanical work. The analysis of the heat production attempted on preceding pages, however, even though only approximate, clearly shows that a considerable portion of the increase in heat production is due to other causes. Roughly, from 9 to 17 per cent of the increase is computed to have had its source in the methane fermentation, while from 3 per cent to as much as 30 or 40 per cent appears to have been due to increased muscular activity while standing. The "remainder" may be regarded as consisting of the mechanical work of digestion plus the stimulus which the feed exerted upon the general metabolism of the animal. How large the latter factor is we have no means of determining, but apparently it is not inconsiderable.

It would seem that the energy expended in peristalsis can not be widely different per kilogram for the different classes of feeding stuffs. On the other hand, the work of mastication and rumination has been shown to be distinctly greater for the coarse feeds. On the basis of Paechtner's (39) and of Dahm's (17) experiments on cattle it may be roughly estimated at 100 Calories per kilogram for hay. Zuntz and Hagemann (52) found the work of masticating oats by the horse to be 28 per cent of that required for hay. On this basis an expenditure by cattle of approximately 28 Calories per kilogram of concentrated feeds, may be estimated. If these amounts are subtracted from those shown in the last column of Table XI, the following approximate figures are obtained per kilogram of dry matter consumed for the work of peristalsis plus the food stimulus to the general metabolism:

COARSE FEEDS	Calories.	CONCENTRATES	Calories.
Timothy hay	404	Maize meal	878
Clover hay (experiment 179)	346	Hominy chop	1,152
Mixed hay	685	Wheat bran	915
Alfalfa hay	802	Grain mixture No. 1	803
Maize stover	775	Grain mixture No. 2	872

Whether the expenditure of energy in peristalsis in cattle is as small as it appears from recent investigations to be in man and in the carnivora it is impossible to say, but one can hardly avoid the impression that the considerable differences shown by the foregoing figures, and

especially the generally higher results for the concentrates, indicate that the direct stimulation of metabolism is a large factor.

It appears, then, that while the mechanical work required for the digestion of concentrates is somewhat less than that necessary in case of coarse fodders, this difference is more than compensated for by other factors, so that on the whole fully as great an increase in the heat production is caused by the consumption of the concentrates. As a class, concentrates are superior to coarse fodders, not because their consumption involves a less expenditure of energy, but because they contain more metabolizable energy, so that more remains available for body use after that expenditure has been met.

DIFFERENCES BETWEEN FEEDING STUFFS

But while our results do not show the existence of as great differences between the two great classes of feeding stuffs in their effects on the energy expenditure of the body as seems to have been at times assumed, they nevertheless reveal distinct differences even between feeding stuffs of the same class. Thus, among the hays (if the results of experiment 179 for clover hay are accepted) a regular increase is found in the total energy expenditure from timothy hay with an average of 782 Calories through mixed hay and clover hay up to alfalfa with an average of 1,169 Calories. Apparently the legumes cause a distinctly greater increase in the metabolism than the Poaceae (Gramineae). In the case of red clover, the difference, according to the meager results obtained, appears to result chiefly from a stimulation of the metabolism due to standing. With alfalfa, on the contrary, the increase in the standing metabolism is not materially greater than in the case of timothy hay, while that due to fermentation is somewhat less. The chief difference between the two seems to lie either in their effect upon the work of peristalsis or in the degree to which they stimulate the general metabolism. One can hardly doubt that the latter is the chief cause and is naturally inclined to associate it with the higher percentage of protein in the legumes. That other causes may also be operative, however, is indicated by the result on maize stover, which is nearly as high as in the case of alfalfa and shows a similar distribution among the several factors.

Among the concentrates there may be noted particularly the marked effect of maize in both the two not very satisfactory experiments in noticeably increasing the standing metabolism. This result is of special interest in view of Zuntz and Hagemann's observations (52, p. 259) on the stimulating effect of maize upon the metabolism of the horse, which were also made on the standing animal, although no increase in the minor muscular activity is reported. Grain mixture No. 1, containing 43 per cent of maize meal, likewise showed a similar effect, although with grain mixture No. 2, containing 60 per cent of maize, it was much less marked, possibly on account of the lower content of protein (12.5 as compared with 17.5 per cent). The increases caused by wheat bran

and by hominy chop, on the other hand, appear to have affected chiefly the metabolism of the animal when lying.

INDIVIDUAL DIFFERENCES

Attention was called on pages 460–461 to the existence of individual differences in the effect of the feed on the ratio of the standing to the lying metabolism. These differences seem in some instances to extend also to other factors of the total heat increment. While the single results are more or less variable, this fact seems to be brought out clearly in the averages. The most striking example is afforded by the animals A and B in experiments 190, 200, and 207, for which the following averages may be computed, showing the heat increments per kilogram of feed to have been distinctly greater with steer B than with steer A. This is, of course, the converse of the conclusion recorded in an earlier publication (10).

Average heat increments of steers A and B per kilogram of dry matter

Feeding stuff.	Animal. No.	Total increment.	Distribution.			
			Standing 12 hours.	Rising and lying down.	Methane fermentation.	Remainder.
		Calories.	*Calories.*	*Calories.*	*Calories.*	*Calories.*
Timothy hay	A	717	113	6	139	459
	B	890	183	4	126	577
Timothy hay and wheat bran	A	1,066	84	0	140	842
	B	1,288	101	−1	144	1,044
Timothy hay and grain mixture No. 1.	A	1,223	296	7	156	764
	B	1,430	367	1	164	898

No such distinct differences were observed between the other animals, which, however, were all of similar type. While steers C, E, and F showed an increased effect upon the standing metabolism in the order named the difference in the metabolism of the animals when lying shows on the average an approximately equivalent decrease, so that no material difference in the total effect resulted.

SUMMARY

Tables X and XI include the results of all of our experiments which have been so far computed as to permit their discussion. In seeking to derive from the recorded results for the increased energy expenditure consequent upon the consumption of certain feeding stuffs general averages which may, with the reservations made on previous pages, afford a basis for estimating the energy values of classes of feeding stuffs and of mixed rations, a certain degree of freedom of choice and the exercise of the judgment of the experimenters seems warranted. Of our results, those on clover hay in experiment 186 appear to us particularly questionable. In one period the animal did not lie down during the entire 48 hours, while in two other periods the time spent in lying was much less than normal. Furthermore, there was a considerable difference

between the observed and the computed heat production in four out of six periods, although it is true that this difference was not relatively greater than in experiment 190. Whether these facts are in any degree responsible for what seem abnormally low results and for the very large proportion of the heat increment apparently due to stimulation of the standing metabolism, it is hard to say, but the results differ so widely from all the others that we feel justified in rejecting them, pending other experiments, particularly since the total increment observed in experiment 179 agrees very well with that computed on page 478 from Kellner's experiments. Experiment 212 fails to show any significant difference between alfalfa hay and alfalfa meal, and the two have been averaged together. In the case of maize meal it is difficult to decide which, if either, of the discordant results is worthy of most credit. The figure of only 393 Calories per kilogram for the increase of the metabolism of the animal when lying, however, seems so low that we are inclined to attach greater weight to the later experiment. For grain mixture No. 2 we have used the results computed from periods 1 and 2 in the belief that the heat production in period 3 was rendered abnormally high by the restlessness of the animals, owing to the small bulk of their ration (compare p. 461), although the difference is scarcely significant.

In the Möckern experiments Kellner's results on heavy rations of starch appear to be abnormal in that the methane production was not increased, while much starch escaped digestion. Kühn's results were obtained on rations of coarse fodder and starch alone with a nutritive ratio of about 1 : 20, or even wider—i. e., under conditions seldom or never realized in practice. Kellner's average for medium rations, therefore, would appear to correspond most nearly to normal conditions. The results on peanut oil were irregular in several respects, but the rejection of the very high result with ox D seems justified. Of the computed results of Kellner's experiments, as given on page 478, those for the oil meals seem unquestionably too low and have been rejected.

On the foregoing assumptions we have formulated the following averages for the total energy expenditure resulting from the consumption of 1 kg. of the dry matter of the feeds named. It may not be superfluous to call attention again to the fact that these figures are simply general averages, derived in some instances from quite discordant single results, and that, as both our own and the Möckern experiments show, they are subject to very considerable variations in individual cases.

Average energy expenditure per kilogram of dry matter eaten

COARSE FODDERS	Calories.
Timothy hay	782
Red clover hay	962
Mixed hay	980
Alfalfa hay	1,169
"Grass hay"	1,045
Rowen	958
Meadow hay	1,254
Maize stover	1,065
Barley straw	877
Oat straw	1,014
Wheat straw	1,138
Extracted straw	1,160
Clover hay	932

CONCENTRATES	Calories.
Maize meal	1,434
Hominy chop	1,365
Wheat bran	1,177
Grain mixture No. 1	1,327
Grain mixture No. 2	1,141
Beet molasses	988
Starch	1,248
Peanut oil	1,727
Wheat gluten	2,294

III. NET ENERGY VALUES AND THEIR COMPUTATION

The method of estimating the nutritive values of the feeding stuffs consumed by farm animals which has been current for many years may from one point of view be characterized in a broad way as a chemical method. On the basis of the fundamental investigations of Henneberg and Stohmann (21, 22) in the early sixties, it sought to determine the amounts of protein, carbohydrates, and fat contained in feeding stuffs in a digestible form, assuming that the groups thus determined had the same physiological values in the nutrition of herbivora as had the corresponding substances in the food of man and carnivora. It is a well-recognized fact, however, that our information regarding both the qualitative and quantitative composition of feeding stuffs is even yet very meager. Moreover, our knowledge of the physiological functions of their ingredients is even more defective, so that, as Kellner (24, p. 15) points out, the advances in our knowledge of the chemistry of plants have not led to a corresponding increase in our knowledge of their nutritive values and have left the methods for the analysis of feeding stuffs largely untouched.

Kellner appears to have been the first to attempt any practical application of the conception of the feed as a source of energy to the body. In 1880, in his investigations upon the relations between muscular activity and metabolism in the horse (23), he determined the additional amount of work which the animal was able to perform as a result of the addition to his rations of starch and of fat. He expressed his results in terms of the percentage of the energy of the starch or fat which was recovered as useful work and called attention to the desirability of determinations of the heats of combustion of nutrients and feeding stuffs. Sixteen years later, after Rubner (40, 41) had published his fundamental work on the replacement values of nutrients and Zuntz and his associates (30, 54) had begun their investigations on the metabolism of the horse from the standpoint of energy, Kellner was able to return to the subject and undertake those extensive investigations with cattle (cited on previous pages) upon which he based his well-known method of comparing feeding stuffs on the basis of their so-called starch values. These are in reality energy values, and, so far as they are the results of direct determinations, they were obtained by substantially the same general experimental methods used in our own investigations, although direct determinations of the heat production were not included.

VALUES DIRECTLY DETERMINED

The net energy value of a feeding stuff, as stated in the introductory paragraphs, is the energy which remains after deducting from its total chemical energy the two classes of losses which have been discussed in the first two sections of this article—viz, the losses of chemical energy

in the excreta and the increased heat production consequent upon the consumption of the feed. For example, the alfalfa hay consumed by steer E in experiment 208 contained per kilogram of dry matter 4,408 Calories of chemical energy. From the results reported in Tables III and X its net energy value, computed from the average results of periods 4, 5, and 6, is as follows:

Net energy value of alfalfa hay per kilogram of dry matter

	Calories.	Calories.	Calories.
Total chemical energy			4,408
Losses of chemical energy:			
In feces	2,062		
In urine	243		
In methane	266		
Total		2,571	
Increased heat production		1,202	
Total losses			3,773
Net energy value			635

Computed in practically this way, by subtracting from the gross energy the average losses of chemical energy recorded in Table IV and the average energy expenditure consequent upon the consumption of the feeding stuff as given on page 482, the average net energy values of the feeding stuffs used in these experiments are as follows:

Net energy values of feeding stuffs per kilogram of dry matter

Feeding stuff.	Gross energy.	Losses of chemical energy.	Energy expended in feed consumption.	Net energy values.
	Calories.	*Calories.*	*Calories.*	*Calories.*
Timothy hay	4,518	2,664	782	1,072
Red clover hay	4,462	2,461	962	1,039
Mixed hay	4,393	2,479	980	934
Alfalfa hay [a]	4,372	2,451	1,169	752
Maize stover	4,332	2,380	1,065	887
Maize meal	4,442	1,115	1,434	1,893
Wheat bran	4,532	2,021	1,177	1,334
Grain mixture No. 1	4,685	1,621	1,327	1,737
Grain mixture No. 2	4,609	1,620	1,141	1,848
Hominy chop	4,709	1,187	1,365	2,157

[a] Includes alfalfa meal.

Kellner's results when put into the same form are as follows:

Net energy values of feeding stuffs per kilogram of dry matter: Kellner's results

Feeding stuff.	Gross energy.	Losses of chemical energy.	Energy expended in feed consumption.	Net energy values.
	Calories.	*Calories.*	*Calories.*	*Calories.*
Meadow hay	4,433	2,260	1,254	919
Oat straw	4,436	2,848	1,014	574
Wheat straw	4,444	3,062	1,138	244
Extracted straw	4,147	1,013	1,160	1,974
"Grass hay" [a]	.....	.....	1,045	803
Rowen [a]	.....	.....	958	747
Barley straw [a]	.....	.....	877	747
Clover hay [a]	.....	.....	932	811
Starch	4,152	1,101	1,248	1,803
Peanut oil	9,457	4,165	1,727	3,565
Wheat gluten	5,579	1,974	2,096	1,509
Beet molasses	3,743	945	988	1,810

[a] As estimated on page 478.

Very striking is the relatively low value for alfalfa hay, due in part to somewhat large losses in the excreta but chiefly to its marked effect in stimulating the metabolism. It is needless to add that this loss does not affect its special value as a source of protein, but as a source of energy it appears to have been distinctly inferior to timothy hay or even to maize stover.

APPLICATION OF RESULTS TO OTHER FEEDING STUFFS

It is obviously impracticable to apply the laborious methods of respiration and calorimeter experiments to all the vast number of feeding stuffs now in use. It is necessary to select a few typical representatives of different groups and to endeavor to apply the results obtained as well as possible to other similar materials. This Kellner sought to do in his later and as yet unpublished experiments. In the practical application of his results, however, Kellner failed to free himself from the older point of view. Aside from what seems to us the unfortunate and unnecessary concession to established usage involved in expressing energy values in terms of matter, he approached the whole problem, as was quite natural, along the lines of the prevailing chemical methods. Determining first the net energy values of the simple nutrients, he applied these values to the digestible nutrients of feeding stuffs and found that in most cases the resulting energy values were materially higher than those obtained by direct experiments on animals. In the case of coarse fodders this deficit in the observed energy values was found to be approximately proportional to the total content of crude fiber, and by subtracting from the computed energy value 1.36 Calories per gram of total crude fiber results were obtained corresponding fairly well to those directly observed.

For finer materials like chaff, presumably requiring a less expenditure for mastication, 0.70 Calorie per gram of total crude fiber is deducted. For green forage containing 16 per cent or more of crude fiber the same deduction is made as for dry forage and for that containing 4 per cent or less of crude fiber, the same as for chaff, while between these limits a sliding scale is used (24, 1905, p. 593–594). For concentrates a factor (Wertigkeit) is estimated from the direct results on similar feeds by which the energy value computed from the digestible nutrients is multiplied to obtain the actual value.

The method of computation just outlined is not only somewhat complicated but is essentially based on the older view which regarded the feed in the light of a source of matter to the body. The digestible protein, carbohydrates, and fat are still the basis of the calculation, although certain more or less empirical corrections are applied to their computed effects. The energy content of a feeding stuff, however, is just as definite a quantity as its content of protein, carbohydrates, or fats, and it is entirely possible to trace the distribution of that energy in the body quite independently of any knowledge of the chemical composition of the materials. Not only so, but we believe that in discussing energy values there are distinct advantages as regards simplicity, and perhaps also as regards accuracy, in cutting loose entirely from the conventional data regarding chemical composition and digestion coefficients, as has been done in reporting our experiments on preceding pages, and in dealing directly with quantities of energy.

In making this statement we would by no means be understood to stigmatize comparisons based on chemical methods as either valueless or superfluous. The problems of nutrition are too complex and too difficult for us to refuse any light that can be thrown on them by any method, and the energy relations touch only one phase of them. The point is that in whatever degree their energetic aspects can be separated from their chemical aspects, to that extent we possess two independent methods of approach to them.

COMPUTATION OF NET ENERGY VALUES

The computation from the results of metabolism experiments or from the data of ordinary feeding tables in the manner just indicated of the net energy value of a feeding stuff which has not been the subject of direct experimental investigation with the respiration apparatus or calorimeter may be made a comparatively simple matter. The net energy value is equal to the metabolizable energy minus the energy lost as heat. It was shown on pages 450–451 that the metabolizable energy may be determined experimentally without special difficulty and with a good degree of accuracy by means of the ordinary metabolism experiment in which the energy of the feed, feces, and urine is directly determined and that

of the methane estimated from the amount of carbohydrates digested. When this is not practicable, it was further shown that the metabolizable energy may be estimated from the total digestible organic matter by the use of the factors given on pages 451–453. In one or other of these ways it is not difficult to compute approximately the metabolizable energy of the more common feeding stuffs, while the subtraction from this of the average energy expenditure due to feed consumption will give the net energy value. To illustrate, E. W. Allen,[1] gives the following data for average alfalfa hay, oat straw, and wheat bran:

Percentage of dry matter and digestible food ingredients of feeding stuffs

	Alfalfa hay.	Oat straw.	Wheat bran.
Total dry matter	91.6	90.8	88.5
Digestible:			
Protein	10.58	1.20	12.01
Carbohydrates	37.33	38.64	41.23
Fats	1.38	0.76	2.87
Total digestible	49.29	40.60	56.11

The sum of the digestible protein, carbohydrates, and fat equals, of course, the total digestible organic matter, irrespective of its chemical composition. Each gram of digestible organic matter, according to the averages on pages 451–453, would contain 3.5 Calories of metabolizable energy in the coarse fodders and 3.9 Calories in the bran. The average losses of energy in heat production per kilogram of feed would be the amounts shown on page 482 reduced to the average water content of the feed, as follows:

Alfalfa hay ..... 1,169×0.916=1,071 Calories.
Oat straw ..... 1,014×0.908= 921 Calories.
Wheat bran ..... 1,138×0.885=1,007 Calories.

The computation of the net energy values is therefore as follows:

Alfalfa hay (3.5 Calories×492.9)−1,071 Calories=654 Calories per kilogram=29.7 T. per 100 pounds.
Oat straw (3.5 Calories×406.0)−921 Calories=500 Calories per kilogram=22.7 T. per 100 pounds.
Wheat bran (3.9 Calories×561.1)−1,007 Calories=1,181 Calories per kilogram=53.6 T. per 100 pounds.

The methods of computation just illustrated are perhaps open to the charge of being to a degree summary and empirical. The idea of basing such computations on the energy values of the single ingredients may be fundamentally more scientific, but unfortunately at present it is an impracticable ideal on account of our deficient knowledge of the chemistry of feeding stuffs and of the physiological values of their ingredients. While investigation along both these lines is highly important and desirable, yet for a long time to come the data on which to base the practice of stock feeding will have to be obtained by more direct even

[1] Allen, E. W., The feeding of farm animals. U. S. Dept. Agr. Farmers' Bul. 22 (rev.), p. 8–9. 1901.

if less fundamental methods. Kellner's scheme recognizes this fact and his deduction for crude fiber and his factors for relative values (Wertigkeit) are at bottom simply a method of applying the aggregate net results on typical feeding stuffs to other materials. The method here proposed seeks to do exactly the same thing more directly and simply, relating the energy content and the necessary deductions to the total dry matter or total digestible matter of the feeding stuff, independently of its chemical composition. It is true that the data for so doing are somewhat meager, but, except as Kellner has utilized unpublished data in the formulation of his tables, they are just as abundant in the one case as in the other. It is greatly to be regretted that Kellner's results have not yet been published in full. When they become available they will doubtless greatly broaden the basis for such computations.

SUMMARY

There are reported the results of 76 experiments with the respiration calorimeter upon nine steers in which the balance of matter and of energy was determined.

The losses of feed energy from the animal are of two classes: (1) Losses of unused chemical energy in the feces, urine, and methane; and (2) losses in the form of heat due to the increased metabolism consequent upon the ingestion of feed.

(1) LOSSES OF CHEMICAL ENERGY.—The losses of energy in methane and urine were relatively greater on light than on moderately heavy rations.

Neither the losses of energy in the feces nor the total losses showed a distinct relation to the amount of feed consumed.

Individual differences between animals had no very material influence on the losses of chemical energy.

The losses of energy in methane may be computed approximately from the amount of total carbohydrates digested.

The metabolizable energy per kilogram of digested organic matter showed but slight variations within the same class of feeding stuffs.

(2) LOSSES OF HEAT CONSEQUENT UPON FEED CONSUMPTION.—The heat production is notably greater during standing than during lying, and the difference is greater on heavy than on light rations.

The increment of heat production during standing is affected by the individuality of the animal and by the kind of feed consumed.

An approximate partial analysis of the heat production of the animal into its principal factors is attempted.

The average energy expenditure consequent upon the consumption of 1 kg. of dry matter is reported for 11 different feeding stuffs.

The expenditure of energy arising from the consumption of the coarse feeds is not on the whole materially greater than in the case of the concentrates.

The increased muscular work of the digestive organs appears to be a relatively small factor of the increased heat production.

A scrub steer showed a somewhat greater increment of metabolism consequent upon feed consumption than did a pure-bred beef animal.

(3) NET ENERGY VALUES.—A summary of the average net energy values obtained in these experiments for 11 different feeding stuffs is given.

A simple method is outlined for computing net energy values, in the absence of direct determinations, from metabolism experiments or from the data of ordinary feeding tables.

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AIR AND WIND DISSEMINATION OF ASCOPORES OF THE CHESTNUT-BLIGHT FUNGUS

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HISTORICAL INTRODUCTION

Wind dissemination of the chestnut-blight fungus (*Endothia parasitica* (Murr.) And.) was first suggested by Murrill (13) [2] in 1906, although he apparently had only the pycnospores in mind, as is shown by the following quotation:

> Later the fruiting pustules push up through the lenticels and give the bark a rough, warty appearance; and from these numerous yellowish-brown pustules millions of minute summer spores emerge from day to day in elongated reddish-brown masses to be disseminated by the wind and other agencies, such as insects, birds, squirrels, etc.

A few years later, in a discussion of the means of spreading the disease, Hodson (9) says:

> Wind is probably the principal agency, but the spores are no doubt carried by animals, birds, insects, and by shipment of infected material.

He also cited some observations to substantiate the wind-dissemination theory, but it was not brought out clearly whether he had in mind the ascopores or the pycnospores only. A similar opinion is expressed by Mickleborough (12) a little later. After speaking of both the ascopores and the conidal, or summer, spores, he states:

> The minute spores are carried by the wind, on the feathers of birds, and the fur of squirrels.

Referring to the spore horns, Mickleborough writes:

> These threads are dissolved and washed away by the rain and the spores are blown about by the wind.

There are two possible ways in which pycnospores might be disseminated by the wind: First, by the direct transport of spore horns or small fragments of these structures; second, by the transport of dust particles bearing spores previously washed down by rains.

Fulton (4) reports experiments which indicate that the former method of transport of pycnospores is of little importance in the spread of the disease. He concludes his discussion of this topic with the following statement:

> It seems likely the detachment was largely of small bits of the tendrils made up of large numbers of spores, and that these are too heavy to be carried great distances;

[1] The writers received valuable assistance in this work from Mr. R. C. Walton, also an agent, Investigations in Forest Pathology.

[2] Reference is made by number to "Literature cited," p. 525–526.

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and suggests that under natural conditions infection may be spread short distances by the wind.

The second possibility is brought out by Metcalf and Collins (11), as may be noted in the following quotation:

As both kinds of spores appear to be sticky, there is no evidence that they are transmitted by wind except where they may be washed down into the dust and so blown about with the dust.

While it has not yet been demonstrated that pycnospores are carried in this way, the tests of Heald and Gardner (7) on the longevity of pycnospores in soil give added plausibility to the theory, since these spores were found to persist in the soil between periods of rain and were able to withstand complete desiccation in the laboratory for months.

Attention was first directed to the strong probability of wind dissemination of ascopores by Rankin (14), who reported their forcible expulsion. In a later report the same writer (15) makes the following statement:

Under moist conditions the ascospores are shot forcibly out in the air where they can be caught up by the wind and carried for a considerable distance. The speaker found ascopores being shot from mature pustules during every rainy period last summer. * * * The question at once arises, Why could not these ascospores once shot into the air be carried long distances and, owing to their abundance, cause a large majority of the infection?

After carrying out field experiments during the summer of 1912, Rankin (16), referring to ascospores, says:

They are shot out in vast numbers with every rain during the summer and are carried by the wind.

Detailed field work on dissemination was carried out by Anderson (1) and his assistants for the Pennsylvania Chestnut Tree Blight Commission (2). These publications confirm the statement of Rankin that expulsion of spores takes place only when the pustules are moist. The seasonal duration of shooting under natural conditions was not determined, as the field tests were confined to the month of August. Under artificial conditions in the laboratory, the time required for moistened bark bearing perithecia to begin the expulsion of spores was determined, the shortest time recorded being three minutes.

The duration of the shooting period following a rain was determined by artificial tests in either the field or laboratory, performed by soaking the specimens or drenching cankers with water. The maximum duration recorded was five hours and two miniutes. While these tests under artificial conditions gave suggestive results, they were not necessarily a reliable indication of what would happen under natural conditions.

It was also determined that bark kept constantly moistened continued to expel spores for a maximum period of 25 days, and the point was emphasized that no continuous rainy weather would be longer. The fact that ascospores expelled during a rain would be washed down to

the ground without being carried any appreciable distance is not mentioned. Since they germinate at once in rain water, the great bulk of such spores would be lost for anything but very local infections. The really important point would appear to be the length of time shooting continues after a rain ceases, for at that time the conditions of the atmosphere would be such as to favor a wider dissemination. This question does not seem to have been satisfactorily answered. The data given on height and horizontal distance of projection, as well as the rate of expulsion, certainly indicate the importance of wind transport of spores following rainy periods.

The spore content of the air was studied by means of aspirator tests and exposure plates. In this work, carried out during dry weather, Anderson and his assistants failed to get positive results under natural conditions in the field. They report the use of over 100 exposure plates and tests of 500 liters of air without finding a single spore of the chestnut-blight fungus. Tests made of aspirated air and by exposure plates gave positive results, however, when the cankers were artificially drenched with water. For the aspirator tests the horizontal distances of the aspirator opening from the canker varied from 2 inches to 5 feet (?) and the maximum vertical distance was 22 feet.

The tests made by exposing agar plates under artificial conditions in the field again pointed to the probability of wind dissemination, but one is forced to admit that they were not conclusive, since the conditions were so different from the natural in that the cankers were drenched with water artificially instead of waiting for a rain. The results with exposure plates may be summed up as follows: No spores of the chestnut-blight fungus were obtained under natural conditions in the field during dry weather; by the use of artificially drenched cankers spores were obtained at distances varying from 1 inch to 51 feet, with very few at the maximum distance.

The final and most conclusive argument in favor of wind dissemination in the minds of the authors cited was afforded by inoculations made by offering an opportunity for wind-borne spores to be introduced into wounds. There is little doubt in the minds of the writers of this paper that infection did take place in the way claimed, but it should be pointed out that a covering of cotton would not prevent spores from being washed into the wounds by rains (6). A fairly compact mass of cotton has been shown to retain but few of the pycnospores present in water passing through it. It must therefore be admitted that, under the conditions of the experiments reported, infection by spores washed down by rains was one of the possibilities.

It is interesting to note in this connection that Kittredge (10), as a result of field observations on the spread of the disease around a center of infection, arrives at the following conclusion:

> The location of infected trees in partially infected groups of sprouts shows that wind is not the prime factor in the distribution of the spores.

The author admitted, however, that the observations reported were rather meager in support of this conclusion.

PURPOSE AND SCOPE OF PRESENT WORK

Since most of the previous work on wind dissemination of the chestnut-blight fungus which yielded positive results was done under artificial conditions, it was the aim of the present writers to study the problem under absolutely natural conditions. Briefly stated, the purpose of these tests was to determine whether or not, and if so, to what extent, wind [1] acts as an agent in dissemination of the spores of this fungus. It was also the object of the work herein recorded to ascertain at what particular times under natural conditions spores of *Endothia parasitica* are prevalent in the air, the possible distances transported by the wind, and the kind of spores (whether ascospores or pycnospores).

The locality chosen in which to conduct our tests was a 4-acre plot of native chestnut (*Castanea dentata*) coppice near West Chester, Pa. The trees in this plot ranged from 4 to 8 inches in diameter and all were badly infected with the chestnut blight, many having already succumbed.

In these tests, which covered a period of 36 consecutive days during August and September, 1913, four methods were employed in studying the points in question. To determine the prevalence of spores of *Endothia parasitica* in the air at particular times and places a series of 756 exposure plates was made. The occurrence of ascospore expulsion was detected and its exact period of duration ascertained by the examination of ascospore traps in the shape of object slides supported over perithecial pustules on the trees. The number of spores present in the air was determined quantitatively by the aspirator method. Rather prolonged exposures of water spore traps, consisting of sterile water in dishes, were made to secure additional information as to the kind of spores in the air, the periods of occurrence, and the distance transported.

EXPOSURE-PLATE TESTS

In testing the spore content of the air among diseased trees in the field for the presence of spores of *Endothia parasitica* the exposure of sterile poured plates of chestnut-bark agar proved to be the most satisfactory method. The use of chestnut-bark agar [2] was found advantageous, since this medium inhibits the development of bacterial colonies and retards the growth of rapid-growing fungi, spores of which are

[1] Falck has pointed out the importance of convection currents in the dissemination of ascospores. (Falck, Richard. Über die Luftinfektion des Mutterkornes (Claviceps purpurea Tul.) und die Verbreitung pflanzlicher Infektionskrankheiten durch Temperaturströmungen. *In* Ztschr. Forst- u. Jagdw., Jahrg. 43, No. 3, p. 202–227, 4 fig., 1911.) For this reason we have used the word "air" in the title of the present paper.

[2] Chestnut-bark agar was made according to the following formula: Add 50 gm. of finely chopped or ground air-dry chestnut bark to 1,000 c. c. of distilled water and boil for 15 minutes. Filter through cheesecloth or absorbent cotton and add water to make up to 1,000 c. c. Add 15 gm. of agar and boil until the agar has melted; then cool to 60° C. or under, clear with the whites of two eggs, filter, and sterilize in the autoclave.

present in the air. At the same time the growth of *E. parasitica* on this medium is vigorous and characteristic.

As supports or stations on which to expose the plates, it was found convenient and satisfactory to make use of the numerous large flat-topped stumps scattered throughout the coppice stand of diseased trees. To facilitate the recording of data, all of the stumps used were numbered with crayon and carefully described and located with regard to surrounding trees (fig. 1). Here it may be mentioned, however, that other supports, such as the top rail of a fence or the top of a stake driven into the ground, were used in case of emergency attendant upon certain weather conditions.

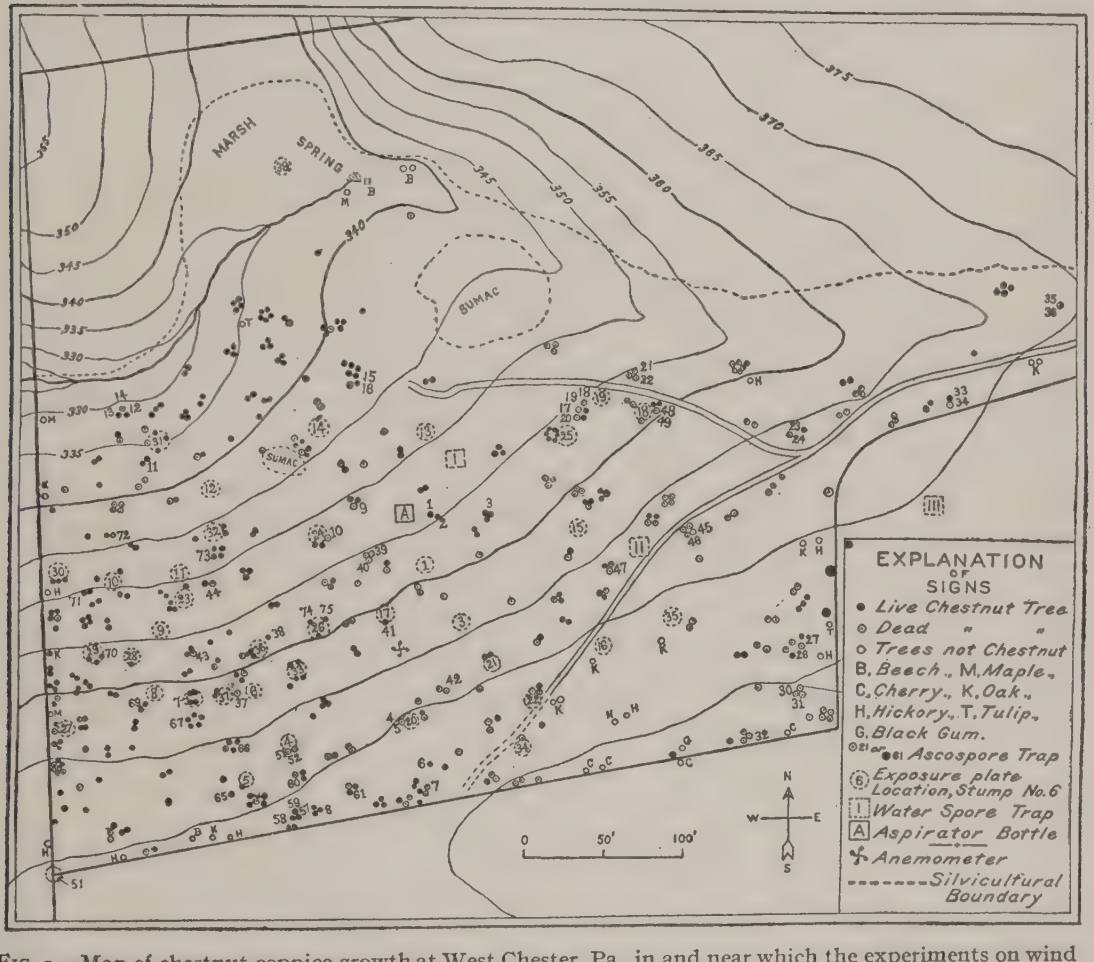

FIG. 1.—Map of chestnut coppice growth at West Chester, Pa., in and near which the experiments on wind dissemination of the chestnut-blight fungus were carried out.

The stumps, rails, and stakes used for this purpose were all of such an age or nature that they were entirely free from lesions of the chestnut blight.

Under conditions of ordinary fair weather the routine followed in making the exposures was similar throughout the tests. Plates were exposed at the rate of one about every half hour during the day, and the average length of exposure was about 5 minutes for each plate during the first 18 days. Then it was found advisable to lengthen the time of exposure, and thereafter 10 minutes, more or less, was the usual time allowed. Wind direction determined what stations were utilized each day, since an

effort was usually made to expose plates at stations where there were many diseased trees to the windward.

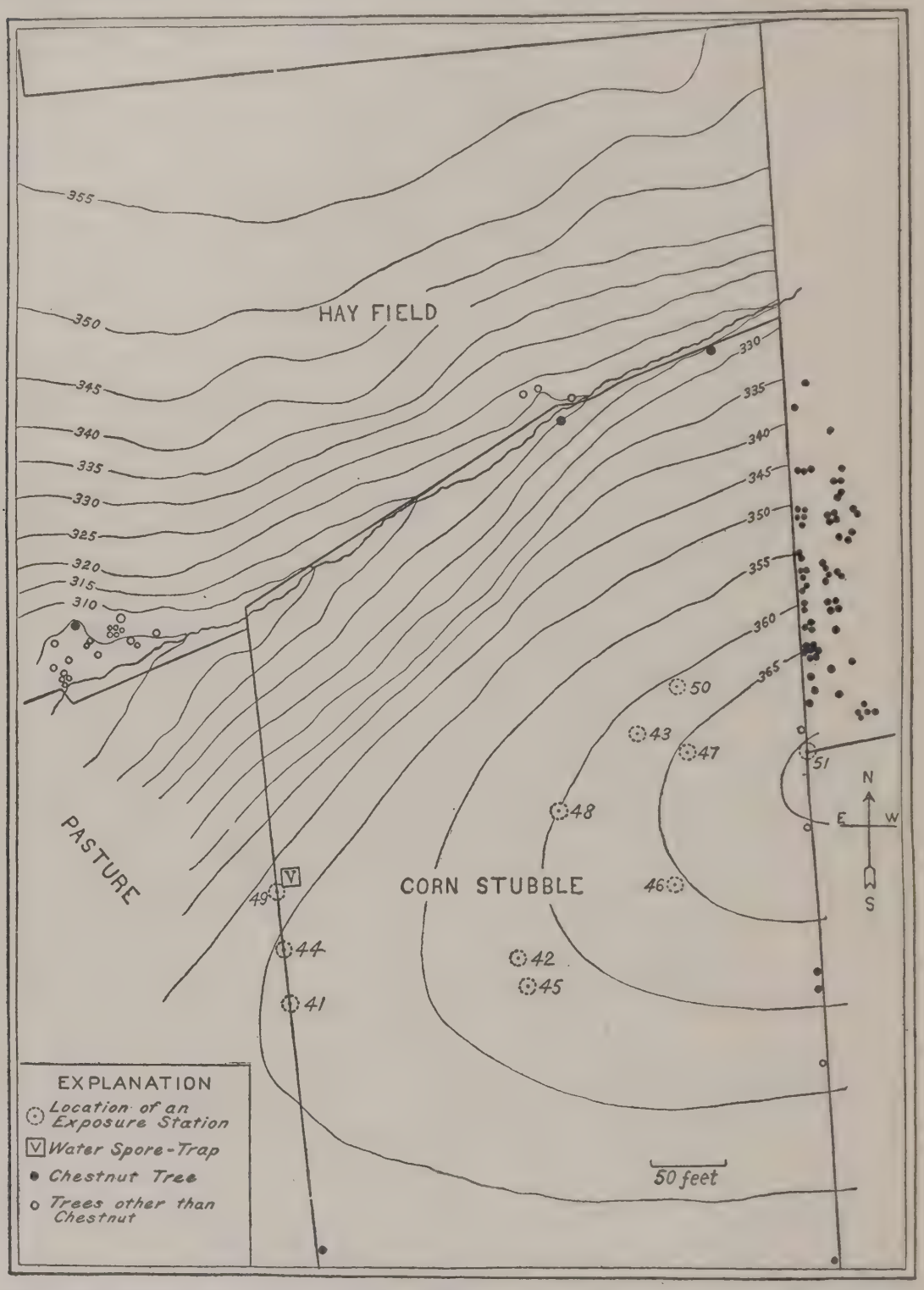

FIG. 2.—Map showing the location of some of the important outlying exposure-plate stations. Station 51 is at the corner of the plot represented in figure 1.

During wet weather the routine was often varied considerably, especially just after the cessation of a rain. At such times plates were often exposed in more rapid succession, even to the extent of exposing several in different locations at about the same time.

An anemometer was erected in the experimental plot, and from the successive readings of this instrument the wind velocities were computed. Continuous records of temperature were secured by means of a thermograph located in a standard instrument shelter near the plot, and by use of a rain gauge the exact rainfall in inches was determined. As complete data as possible were also secured relative to the exact duration of all rains.

In describing and locating the stations used for exposure plates, measurements were made to the nearest diseased trees and to the nearest lesions, the horizontal distance being recorded. To supplement the description, detailed topographic maps were made, showing the location of each station (figs. 1 and 2).

The exposed plates were incubated at room temperature, and two records were usually taken. First, at the end of three days after exposure all fungous and bacterial colonies visible were marked and counted, and those suspected of being *Endothia parasitica* were especially noted. After six or seven days of incubation the final record on each plate was taken. This included the total number of fungi, the number of bacterial and yeast colonies, and the number of colonies of *E. parasitica*, if any were present. In case of doubt as to the identity of the latter, owing to crowding by other colonies, transfers were made to 3 per cent dextrose agar, on which medium the growth of this fungus is even more characteristic than on chestnut-bark agar.

The results obtained in the exposure-plate tests are presented in a somewhat summarized form in Tables I and II.

TABLE I.—*Summary of exposure-plate tests at West Chester, Pa., in 1913, giving number of fungous colonies caught*

Date of cultures.	Number of plates exposed.	Length of exposure for each plate.	Total time represented.	Rainfall.	Number of fungous colonies in any plate.		Total number of fungous colonies.	Total number of colonies of Endothia parasitica.
					Maximum.	Minimum.		
		Minutes.	*H. m.*	*Inches.*				
Aug. 19..	18	5 to 6	1 39	0.40	81	3	332	1
20..	22	6 to 10	2 19¼	0	24	1	227	0
21..	19	5 to 7	1 48½	0	26	0	168	0
22..	20	1½ to 7½	1 44	0.25	35	2	340	0
23..	25	4 to 8	2 19		28	1	256	0
24..	17	4½ to 6	1 28¼	0	47	1	206	0
25..	19	4 to 6	1 34	0	75	5	419	0
26..	20	4 to 7	1 48½	0	95	8	574	0
27..	28	4 to 9⅓	2 44½	0.175	70	4	446	94
28..	18	4 to 6½	1 32	0	20	2	196	0
29..	19	4 to 5½	1 30½	1.10	51	1	309	0
30..	22	5 to 6½	1 57¼		146	0	363	0
31..	20	4 to 7	1 48	0	32	1	261	1
Sept. 1..	19	5 to 6½	1 44	0	12	1	122	0
2..	19	4 to 6	1 37	0	39	3	213	0
3..	19	5 to 6½	1 37½	0	10	1	68	2
4..	18	5	1 30	0	48	5	245	1
5..	18	5 to 6	1 31	0	40	0	179	1

TABLE I.—*Summary of exposure-plate tests at West Chester, Pa., in 1913, giving number of fungous colonies caught*—Continued

Date of cultures.	Number of plates exposed.	Length of exposure for each plate.	Total time represented.	Rainfall.	Number of fungous colonies in any plate.		Total number of fungous colonies.	Total number of colonies of Endothia parasitica.
					Maximum.	Minimum.		
		Minutes.	*H. m.*	*Inches.*				
Sept. 6..	18	5 to 10½	2 14¾	0	6	0	39	0
7..	19	7 to 11¾	3 4¾	0.37	22	1	186	0
8..	27	7¼ to 12	4 16		15	1	195	9
9..	18	6 to 11½	2 23	0	31	2	194	2
10..	18	8 to 11¾	2 50¼	0	31	0	138	0
11..	18	9½ to 14¼	3 19¼	0	25	0	118	0
12..	18	5 to 14	3 12½	0.095	41	1	121	0
13..	19	7 to 13	3 5¼		73	2	332	0
14..	18	9 to 11¼	3 9½	0	20	2	126	0
15..	18	9½ to 11	3 6	0	20	6	202	0
16..	19	9½ to 27	3 34½	0	28	0	251	0
17..	17	9½ to 15½	3 13¾	0.26	36	2	221	[a] 2
18..	28	9¾ to 13½	5 15¼	0.68	67	1	539	55
19..	26	10¼ to 17	5 24½	0.09	400	1	814	7
20..	28	9¼ to 18	6 13¾	0.43	23	1	194	90
21..	25	3½ to 20	5 52½	0.10 0.73	160	5	1,187	160
22..	36	6½ to 30	9 50		60	0	494	2
23..	24	12¼ to 23	6 36	0	30	0	261	0

[a] From wind-blown bark fragments.

TABLE II.—*Detailed record of all exposure plates in which spores of Endothia parasitica were caught at West Chester, Pa., in 1913*

Plate No.	Date of exposure.	Rainfall.	Time elapsed since cessation of rain.	Length of exposure.	Horizontal distance to nearest blight lesion.	Number of spores of Endothia parasitica caught.
		Inches.	*D. h. m.*	*Minutes.*	*Feet.*	
4008	Aug. 19	0.4	6+ 0	5	[a] 1½	1
4376	Aug. 27	.12	42	9⅓	4½	1
4382	.....do	.12	1 44	6	15½	3
4383	.....do	.055	18	6½	15½	16
4384	.....do	.055	20	6	11	21
4385	.....do	.055	22	8	25	33
4386	.....do	.055	33	5	4½	20
4460	Aug. 31	1.10	1 10+ 0	5	[a] 2	.1
4509	Sept. 3	1.10	5 0 0	5	[a] 2	2
4529	Sept. 4	1.10	5 0 0	5	[a] 2	1
4553	Sept. 5	1.10	6 0 0	5	[a] 2	1
4599	Sept. 8	.37	5+ 0	10½	11	2
4600	.....do	.37	5 1	11½	14	1
4602	.....do	.37	5 21	12	[a] 1	1
4606	.....do	.37	7 0	9¼	[a] 2	1
4609	.....do	.37	8 30	12	[a] 1½	1
4610	.....do	.37	9 0	10	[a] 2½	1
4617	.....do	.37	13 0	8	[a] 2	1
4619	.....do	.37	14 0	9	[a] 2	1
4626	Sept. 9	.37	1 8 0	6	[a] 2	1
4627	.....do	.37	1 8 30	8½	[a] 4½	1

[a] Stumps more or less overhung by diseased sprouts.

TABLE II.—*Detailed record of all exposure plates in which spores of Endothia parasitica were caught at West Chester, Pa., in 1913*—Continued

Plate No.	Date of exposure.	Rainfall.	Time elapsed since cessation of rain.	Length of exposure.	Horizontal distance to nearest blight lesion.	Number of spores of Endothia parasitica caught.
		Inches.	*D. h. m.*	*Minutes.*	*Feet.*	
4772	Sept. 17	0.095	4 0 0	10	[a] 2½	1
4784	do	.095	4 0 0	10½	[a] 2	[b] 1
4787	Sept. 18	.26	1 51	9¾	15½	11
4788	do	.26	1 54	10	16	16
4789	do	.26	2 0	10	4½	9
4790	do	.26	2 19	13	11	4
4791	do	.26	2 35	11	7	5
4792	do	.26	2 48	11	15½	2
4793	do	.26	2 54	12	[a] 4½	2
4795	do	.26	3 25	11	15½	2
4796	do	.26	3 34	11	17½	1
4797	do	.26	3 58	10¼	16	1
5006	do	.26	9 14	13½	[a] 1	1
5010	do	.26	11 19	11	[a] 2	1
5018	Sept. 19	.68	1 23	11¼	4½	2
5020	do	.68	2 7	12½	27	1
5027	do	.68	6 4	11½	[a] 2	1
5029	do	.68	7 8	12¾	[a] 5	1
5033	do	.68	10 5	10¼	[a] 5	1
5037	do	.68	12 7	13½	27	1
5041	Sept. 20	.09	1 55	16	217	10
5042	do	.09	2 0	13	195	7
5043	do	.09	2 1	14	110	4
5044	do	.09	2 8	12¾	27	22
5045	do	.09	2 19	12¼	85	11
5046	do	.09	2 23	15	180	7
5047	do	.09	2 25	15	237	10
5048	do	.09	2 45	13½	7	6
5049	do	.09	3 12	11½	11	7
5050	do	.09	3 37	13	11	3
5051	do	.09	3 45	16¾	86	1
5052	do	.09	4 29	10	4½	1
5053	do	.09	5 14	13½	5	1
5069	Sept. 21	[c] .35	0	14½	19	12
5070	do	.35	5	10½	17½	20
5071	do	.08	15	20	17½	62
5072	do	.08	17	19	19	24
5073	do	.08	44	13½	17½	19
5074	do	.08	56	15¼	14	1
5075	do	.08	1 1	12	7	2
5087	do	[c] .07	20	14½	19	20
5098	Sept. 22	.73	6 0	16	77	1
5102	do	.73	6 30	15	1½	1

[a] Stumps more or less overhung by diseased sprouts. [b] From fragments of bark. [c] Approximately.

To supplement the tables, it may be well to give in chronological order a more detailed record of the actual routine pursued.

A rain occurred the night previous to August 19, and examination of the ascospore traps (see "Ascospore-trap tests") showed that abundant expulsion of ascospores had occurred. But when the first plates were exposed too long a time had evidently intervened since the rain, as no positive results were obtained, except that one colony of *Endothia parasitica* developed in a plate exposed in the early afternoon.

The next two days were fair, and as was expected for these weather conditions, no spores of this Endothia were caught. In the evening of August 22 there was a rain of 0.25 inch; 6 plates, therefore, were exposed early the next morning before the sun had dried the vegetation. Although the ascospore traps gave evidence that expulsion had occurred, no positive results were obtained, which is explained by the fact that again too long a time had elapsed after the rain ceased.

Dry, hot weather now continued until the afternoon of August 27, and the exposure plates yielded no evidence of the presence of spores of *Endothia parasitica* in the air. In the afternoon of August 27, however, two thunder storms occurred, in consequence of which the regular routine was departed from. Tables II and III show the outcome of the tests of this date. After the first storm two sets of plates were exposed in the course of an hour and a half. Two out of the second set yielded colonies of *E. parasitica*. Since the ascospore-trap tests (Tables X and XVII) did not give evidences of expulsion occurring when the first five plates were exposed, negative results were to be expected in those plates, and it is not surprising that only two out of the second set of seven plates yielded colonies of *E. parasitica* when the 19 ascospore traps examined for this particular period showed evidence of expulsion from only one perithecium. The meagerness of these results is partially accounted for by the small amount of rain, rapid drying, and the fact that the perithecia had hardly been wet a sufficient length of time.

TABLE III.—*Record of exposure plates made on August 27, 1913, at West Chester, Pa.*

BEFORE RAIN.

Plate No.	Time.	Length of exposure.	Station No.	Wind.		Number of bacteria and yeasts.	Total number of fungi.	Number of colonies of Endothia parasitica.
				Direction.	Miles per hour.			
		Minutes.						
4359	8.35 a. m.....	4	1	SW.	1.6	2	7	0
4360	9.09 a. m.....	5	3	SW.	1.6	1	8	0
4361	9.32 a. m.....	5	1	SW.	1.6	1	6	0
4362	10.03 a. m....	5	13	SW.	1.6	0	6	0
4363	10.29 a. m....	6	14	SW.	1.6	1	7	0
4364	10.57 a. m....	5	3	W.	1.6	3	9	0
4365	11.34 a. m....	5½	1	W.	1.7	2	19	0
4366	11.57 a. m....	5	1	W.	1.7	0	15	0
4367	1.16 p. m.....	5	1	W.	1.7	0	22	0
4368	1.39 p. m.....	5	13	W.	1.7	0	33	0
4369	2.09 p. m.....	5½	13	W.	1.7	1	13	0
4370	2.45 p. m.....	5	3	W.	1.7	1	7	0

RAIN NO. 1 (0.12 INCH, 3.15 TO 3.28 P. M.)

4371	3.48 p. m.....	7	3	W.	2.4	1	12	0
4372	3.50 p. m.....	6½	15	W.	2.4	0	10	0
4373	3.49 p. m.....	6⅝	13	W.	2.4	0	17	0
4374	3.48 p. m.....	6¾	1	W.	2.4	0	32	0
4375	3.51 p. m.....	7	8	W.	2.4	0	9	0
4376	4.10 p. m.....	9⅓	6	W.	Trace.	0	7	1
4377	4.12 p. m.....	8⅔	11	W.	Trace.	0	8	0
4378	4.14 p. m.....	8⅙	3	W.	Trace.	2	15	0
4379	4.15 p. m.....	8	16	W.	Trace.	0	11	0
4380	4.17 p. m.....	5⅝	15	W.	Trace.	1	9	0
4381	4.40 p. m.....	4	3	W.	Trace.	0	4	0
4382	5.12 p. m.....	6	3	W.	Trace.	1	13	3

TABLE III.—*Record of exposure plates made on August 27, 1913, at West Chester, Pa.*—Continued

RAIN NO. 2 (0.055 INCH, 5.35 TO 5.50 P. M.)

Plate No.	Time.	Length of exposure.	Station No.	Wind.		Number of bacteria and yeasts.	Total number of fungi.	Number of colonies of Endothia parasitica.
				Direction.	Miles per hour.			
		Minutes.						
4383	6.08 p. m.....	6½	3	NW.	1.2	0	30	16
4384	6.10 p. m.....	6	1	NW.	1.2	0	25	21
4385	6.12 p. m.....	8	16	NW.	1.2	2	70	33
4386	6.23 p. m.....	5	6	N.	.7	0	25	20

The second shower on August 27 took place late in the afternoon, and though the precipitation was light, the cumulative effect of this rain upon that of the preceding one caused abundant expulsion of ascospores. The four plates exposed within about half an hour after this shower yielded colonies of *E. parasitica* in such numbers as to prove beyond doubt that the ascospores were at that time very prevalent in the air. The ascospore-trap tests for this period (Tables X and XVII) showed that, although out of the 14 examined only 1 bore any evidence of spore expulsion during the first 15 minutes after the cessation of the rain, 12 out of 14 showed expulsion of ascospores during the time in which the plate exposures were made. The sun had gone down, and the weather conditions following this storm were not conducive to the rapid drying of the bark. The results of this date were the first evidence secured which indicated beyond doubt that ascospores of *Endothia parasitica* are disseminated by wind under natural conditions.

During the dry, hot weather of August 28 evidently no spores of *Endothia parasitica* were present in the air, nor were any detected on August 29. On this date the humidity was high and cloudiness prevailed, accompanied by traces of rain insufficient to cause ascospore expulsion. As there was a rainfall of 1.10 inches in the evening of August 29, several plates were exposed in rapid succession the next morning, but no spores were caught. This failure is attributed to the fact that once more too long a time had passed since the rain ceased, and spore expulsion, though probably abundant in the night, had no doubt ceased long before the first exposures were made. Throughout the following week there was no rain, and no ascopore expulsion occurred at any time.

For the night previous to September 8 a rainfall of 0.37 inch was recorded, the time of cessation being prior to 1.30 a. m. The ascopore traps gave evidence of plentiful spore expulsion. Between 6.27 and 8 a. m. eight exposures were made before the sun had dried the vegetation and while the bark was still wet in places. Three of these plates yielded colonies of *Endothia parasitica*, and as two of them were exposed at stations more or less in the open, it would seem that ascospores were at that time prevalent in the air to some extent. The third plate and also five others exposed at later intervals during the day each yielded one colony of *E. parasitica*.

Of the plates exposed on September 9, a dry, hot day, two in the morning also yielded one colony each of *Endothia parasitica*. During the dry weather of September 10 and 11 negative results were obtained.

Cloudiness prevailed on September 12, with traces of rain insufficient to cause spore expulsion. In the night less than one-tenth of an inch of rain fell, and subsequent examination of the ascospore traps showed that very light ascospore expulsion had occurred. Five plates were exposed before 8 o'clock the next morning, but the bark was dry at the time and no spores were obtained in any of the plates exposed that day. Three days of clear, hot weather followed, and no spores were caught. Of the plates exposed on September 17, two yielded one colony each of the fungus.

In the evening of September 17 a series of rains began, occurring usually in the night. Our most important positive results were obtained from tests made following these rains. Tables II and IV give the results of the plates exposed on September 18. Although the rain ceased before 4 a. m., a heavy fog prevailed in the early morning, there was only a trace of wind, and it was more or less cloudy all day. Because of these conditions the bark of the trees was slow in drying, and examination of the ascospore traps (Tables XI and XVII) showed that abundant spore expulsion had occurred in the night and was still in progress while the first four exposure plates were made. A few of the traps gave evidences of the continuation of expulsion during the time in which the next 11 plates were exposed. Six of these yielded colonies of *Endothia parasitica* in varying numbers, and two exposed much later in the day also showed one colony each.

TABLE IV.—*Record of exposure plates made on September 18, 1913, at West Chester, Pa.*[a]

Plate No.	Time.	Length of exposure.	Station No.	Wind.		Number of bacteria and yeasts.	Total number of fungi.	Number of colonies of Endothia parasitica.
				Direction.	Miles per hour.			
		Minutes.						
4787	5.51 a. m.	9¾	3	NW.	Trace.	4	20	11
4788	5.54 a. m.	10	15	NW.	Trace.	6	58	16
4789	6.00 a. m.	10	6	NW.	Trace.	6	39	9
4790	6.19 a. m.	13	1	NW.	Trace.	(b)	59	4
4791	6.35 a. m.	11	11	NW.	Trace.	(b)	67	5
4792	6.48 a. m.	11	3	NW.	Trace.	1	16	2
4793	6.54 a. m.	12	4	NW.	Trace.	(b)	32	2
4794	7.02 a. m.	10½	16	NW.	Trace.	(b)	23	0
4795	7.25 a. m.	11	3	NW.	Trace.	(b)	30	2
4796	7.34 a. m.	11	13	NW.	0.4	3	8	1
4797	7.58 a. m.	10¼	15	W.	.7	8	11	1
4798	8.04 a. m.	10¾	9	W.	.7	0	2	0
4799	8.29 a. m.	10	17	W.	.7	0	1	0
5000	9.20 a. m.	12¼	1	W.	1.1	0	8	0
5001	9.49 a. m.	11	3	W.	1.8	0	25	0
5002	10.21 a. m.	10¼	6	W.	2.8	5	10	0
5003	10.54 a. m.	12	15	W.	2.8	1	19	0
5004	11.25 a. m.	11	33	W.	3.7	0	12	0
5005	11.51 a. m.	11½	3	W.	3.7	3	13	1
5006	1.14 p. m.	13½	26	W.	2.2	1	17	0
5007	1.44 p. m.	10¼	21	W.	2.8	0	7	0
5008	2.15 p. m.	10½	22	W.	1.8	1	10	0
5009	2.46 p. m.	15	6	W.	1.2	1	12	1
5010	3.19 p. m.	11	33	W.	1.2	0	12	0
5011	3.45 p. m.	11	21	W.	.9	1	16	0
5012	4.16 p. m.	13½	25	W.	.9	0	1	0
5013	4.44 p. m.	11½	23	W.	.2	4	21	0
5014	5.23 p. m.	10¾	1	W.	.1	0	3	0

[a] Rainfall, night previous, 0.26 inch. Time of cessation, prior to 4 a. m. [b] Numerous.

In the night of September 18 a rain of 0.68 inch was recorded, the time of cessation being the next morning before 3.45. Fog again prevailed all day September 19 and a noticeable spray fell until 5.38 a. m. and began again after 2.41 p. m. Ascospore-trap tests (Tables XII and XVII) showed that in 10 out of the 17 traps examined there was spore expulsion after 7.35 a. m. Of the eight plates exposed prior to this time but two yielded colonies of this Endothia. The first three plates were exposed in an open field at considerable distances from the trees (fig. 2, stations 41, 42, and 43) in the same direction toward which the wind was blowing, but no spores of *E. parasitica* were caught. These negative results may be accounted for by the action of the falling mist. Later in the day four exposures at various intervals yielded one colony each of the chestnut-blight fungus.

TABLE V.—*Record of exposure plates made on September 20, 1913, at West Chester, Pa.*[a]

Plate No.	Time.	Length of exposure.	Station No.	Wind.		Number of bacteria and yeasts.	Total number of fungi.	Number of colonies of Endothia parasitica.
				Direction.	Miles per hour.			
		Minutes.						
5041	5.50 a. m.....	16	44	NE.	2.6	0	12	10
5042	5.55 a. m.....	13	45	NE.	2.6	0	9	7
5043	5.56 a. m.....	14	46	NE.	2.6	0	10	4
5044	6.03 a. m.....	12¾	51	NE.	2.6	0	23	22
5045	6.14 a. m.....	12¼	47	NE.	2.6	0	14	11
5046	6.18 a. m.....	15	48	NE.	2.6	0	9	7
5047	6.20 a. m.....	15	49	NE.	2.6	0	11	10
5048	6.40 a. m.....	13½	11	ENE.	2.6	0	6	6
5049	7.07 a. m.....	11½	1	ENE.	2.6	0	9	7
5050	7.32 a. m.....	13	9	ENE.	2.6	0	6	3
5051	7.40 a. m.....	16¾	50	ENE.	2.6	0	6	1
5052	8.24 a. m.....	10	6	ENE.	2.5	0	1	1
5053	9.09 a. m.....	13½	8	ENE.	2.5	0	1	1
5054	9.37 a. m.....	14½	27	ENE.	3.0	5	11	0
5055	10.01 a. m....	14¾	6	ENE.	2.4	0	2	0
5056	10.32 a. m....	9¼	10	E.	2.4	0	3	0
5057	11.09 a. m....	10	29	E.	2.6	0	12	0
5058	11.44 a. m....	11¼	26	E.	2.6	0	6	0
5059	12.08 p. m....	10	10	E.	2.7	0	2	0
5060	1.05 p. m.....	14½	30	E.	2.7	0	2	0
5061	1.35 p. m.....	13½	11	E.	2.5	0	1	0
5062	2.08 p. m.....	14¾	10	E.	2.5	0	19	0
5063	2.36 p. m.....	15½	8	E.	2.7	2	4	0
5064	3.09 p. m.....	13¼	9	E.	2.7	0	1	0
5065	3.42 p. m.....	14	29	E.	2.7	0	2	0
5066	4.06 p. m.....	18	23	E.	2.3	0	4	0
5067	4.41 p. m.....	12¼	12	ESE.	2.7	0	4	0
5068	5.25 p. m.....	12	12	SE.	2.7	3	4	0

[a] Rainfall, night previous, 0.09 inch. Time of cessation, 3.25 to 3.55 a. m.

While there was but 0.09 inch of rain in the night of September 19, very important results were obtained on September 20. Tables II and V give the results secured. Fog prevailed during the entire day, and the bark on the trees dried very slowly. Examination of the ascospore traps to determine the duration of spore expulsion (Tables XIII and XVII) showed that in 8 out of 19 the perithecia were active after 9.18 a. m.

All of the 13 plates exposed previous to this time yielded colonies of *Endothia parasitica*. Eight of these exposures were made in the open field at varying distances south and west of the plot of diseased trees (fig. 2), the wind being from the northeast. The distance relations brought out by these tests are discussed later. Although five ascospore traps showed evidences of the occurrence of spore expulsion after 10.07 a. m., no colonies of this fungus appeared in any of the plates exposed after 9.23 a. m. This indicates that spores were evidently not sufficiently numerous in the air after that time to be detected by the exposure-plate method.

The results obtained on September 21, as shown in Tables II and VI, bring out again the direct relation of rain to wind dissemination. Two plates exposed during a 16-minute interval between showers in the early morning yielded colonies of *Endothia parasitica* in such numbers as to prove without doubt that ascospores were very prevalent in the air at that time. After the second rain, ending at 8.20 a. m., only the five plates exposed within an hour after its cessation yielded colonies of *E. parasitica*, even though 14 out of the 21 ascospore traps examined showed that considerable spore expulsion had taken place after 10 a. m. (Table XI). However, a south wind of increasing velocity prevailed, and at 9.21 the sun appeared, causing a marked rise in temperature, so that the bark dried very rapidly after that time. Furthermore, the higher wind may also have dispersed and scattered the fewer spores expelled thereafter to such an extent that none happened to fall into the exposed plates.

TABLE VI.—*Record of exposure plates made on September 21, 1913, at West Chester, Pa.*

RAIN NO. 1 (ABOUT 0.35 INCH, CEASED 6.24 A. M.)

Plate No.	Time.	Length of exposure.	Station No.	Wind.		Number of bacteria and yeasts.	Total number of fungi.	Number of colonies of Endothia parasitica.
				Direction.	Miles per hour.			
		Minutes.						
5069	6.23 a. m.....	14¼	12	SSE.	2.6	(a)	30	12
5070	6.30 a. m.....	10½	13	SSE.	2.6	(a)	50	20

RAIN NO. 2 (ABOUT 0.08 INCH, 6.40 TO 8.20 A. M.)

5071	8.35 a. m.....	20	13	SSE.	2.6	(b)	70	62
5072	8.37 a. m.....	19	12	SSE.	2.6	2	28	24
5073	9.04 a. m.....	13½	13	SSE.	3.1	1	21	19
5074	9.16 a. m.....	15¼	19	S.	3.1	1	5	1
5075	9.21 a. m.....	12	11	S.	3.1	0	9	2
5076	9.40 a. m.....	15	13	S.	3.1	3	37	0
5077	9.43 a. m.....	13½	37	S.	3.1	1	18	0
5078	10.25 a. m.....	15	13	SSW.	5.8	3	30	0
5079	10.28 a. m.....	16	19	SSW.	5.8	12	80	0
5080	10.46 a. m.....	10	37	SSW.	5.8	0	21	0
5081	11.00 a. m.....	12	14	SSW.	5.8	6	44	0
5082	11.07 a. m.....	13½	37	SSW.	5.8	1	28	0
5083	11.48 a. m.....	16½	12	SSW.	5.8	5	98	0
5084	1.19 p. m.....	15½	13	S.	5.6	0	26	0

[a] Numerous. [b] Few.

TABLE VI.—*Record of exposure plates made on September 21, 1913, at West Chester, Pa.*—Continued

RAIN NO. 3 (ABOUT 0.03 INCH, 1.43 TO 2.11 P. M.)

Plate No.	Time.	Length of exposure.	Station No.	Wind.		Number of bacteria and yeasts.	Total number of fungi.	Number of colonies of Endothia parasitica.
				Direction.	Miles per hour.			
		Minutes.						
5085	2.20 p. m.....	3½	13	S.	6.2	8	160	0
RAIN NO. 4 (ABOUT 0.04 INCH, 2.20 TO 2.50 P. M.)								
5086	2.53 p. m.....	15¾	13	S.	6.2	0	144	0
5087	3.10 p. m.....	14½	12	S.	6.2	0	35	20
RAIN NO. 5 (ABOUT 0.03 INCH, 3.26 TO 4.07 P. M.)								
5086A	4.08 p. m.....	19¾	13	S.	8.0	10	58	0
5087A	4.17 p. m.....	14	37	S.	8.0	3	50	0
5088	4.35 p. m.....	10	11	S.	8.0	4	45	0
5089	4.55 p. m.....	15	12	S.	8.0	6	42	0
5090	5.16 p. m.....	17½	11	S.	8.0	3	38	0
5091	5.33 p. m.....	11	10	S.	8.0	5	20	0

In the afternoon of the same day three light showers occurred, and one plate exposed in the interval after the second of these caught 20 ascospores. After the first of these showers the exposure was cut too short by the recurrence of rain to give a reliable test. It will be seen that none of the six plates exposed during the 1 hour and 36 minutes after the last shower yielded colonies of *Endothia parasitica*, despite the fact that 6 out of 11 ascospore traps examined (Tables XIV and XVII) gave evidence that expulsion had occurred during that period. In explanation it may be stated that the wind had attained a higher velocity at this time and was blowing quite briskly in the open. It is readily conceivable that with such a wind the spores as they were expelled might have been transported with such speed and their numbers dissipated so rapidly that none chanced to fall on the rather small area represented by the exposure plates.

A rather heavy rainfall was recorded on the night of September 21, but it ceased before 12.45 a. m. Examination of the ascospore traps showed that there was abundant spore expulsion during the night, and 5 out of 21 traps gave evidences of the occurrence of expulsion after 7.30 a. m., on September 22 (Tables XV and XVII). Of the 13 plates exposed between 5.56 a. m. and 7.35 a. m. but 2 yielded positive results (Table II). No spores were caught in any of the plates exposed thereafter, even though two ascospore traps bore evidences of the occurrence of light expulsion after 11.24 a. m. The meager results obtained on this date are no doubt due to the long period of time intervening since the cessation of rain the night before.

Clear, hot weather prevailed during September 22 and 23, and no spores were caught.

The relation of the time elapsed since the cessation of rain to the prevalence of ascospores in the air among diseased trees is shown in Table VII.

TABLE VII.—*Relation of the time elapsed since the cessation of rain to the number of spores falling on an area of 1 square foot per minute in 1913 at West Chester, Pa.*

PLATES EXPOSED ON SEPTEMBER 18, 1913

No. of plate.	Time elapsed since cessation of rain.	Number of colonies of Endothia parasitica.	Number of spores of Endothia parasitica falling on an area of 1 square foot per minute.
	H. *m.*		
4787	1 51	11	15.74
4788	1 54	16	22.32
4789	2 ..	9	12.55
4790	2 19	4	4.29
4791	2 35	5	6.34
4792	2 48	2	2.53
4793	2 54	2	2.32
4795	3 25	2	2.53
4796	3 34	1	1.27
4797	3 58	1	1.36
4798	4 4	0	0
4799	4 29	0	0

PLATES EXPOSED ON SEPTEMBER 21, 1913

5071	15	62	43.24
5072	17	24	17.62
5073	44	19	19.64
5074	56	1	0.91
5075	1 1	2	2.32
5076	1 20	0	0
5077	1 23	0	0
5078	2 5	0	0

An examination of these tables shows that on September 18 the spore content of the air decreased more or less gradually during the third and fourth hours after the rain, while on September 21 the spore content decreased very abruptly and no spores were obtained after the first hour following the cessation of the rain. The duration and the abundance of the ascospore expulsion on these dates (Table XI) are seen to have differed likewise, and a comparison of the weather conditions gives the probable explanation, since it was calm and foggy on the 18th and hot and sunny with a brisk wind just following the rain of the 21st. Conditions following the rain on the 18th were such as to prevent rapid drying of the bark, so that spore expulsion continued during a much longer time than on the 21st, when the bark dried rapidly. Furthermore, the brisk wind of September 21 would tend to disperse the spores very rapidly, whereas the comparative calm of September 18 would be favorable to a more prolonged prevalence in the air near their source. In this regard

it should also be noted that on September 20 (Table II), when foggy weather followed the rain, spores were prevalent in the air during at least five hours after the rain had ceased.

A glance at the figures representing the number of spores falling each minute on a surface equal to 1 square foot shows that during periods of one to four or more hours after a rain—in other words, during such time as expulsion continues—healthy trees among diseased ones would be subject to infection, since some of the ascospores would find lodgment upon exposed parts of trunks and branches.

The results obtained in the early morning of September 20 by making exposures in an open field at varying distances from the principal source of spores (figs. 2 and 3) are presented in Table VIII.

TABLE VIII.—*Relation of distance from source of spores to number of spores falling on an area of 1 square foot per minute in 1913 at West Chester, Pa.*[a]

Plate No.	Time.	Distance from source of spores.	Number of spores falling on an area of 1 square foot per minute.
		Feet.	
5044	6.03 a. m.	27	24.07
5045	6.14 a. m.	85	12.52
5046	6.18 a. m.	180	6.51
5042	5.55 a. m.	266	7.51
5047	6.20 a. m.	409	9.30
5041	5.50 a. m.	414	8.71

[a] Plates exposed on Sept. 20, 1913.

These exposures, all made within about half an hour and in the same general direction from the plot of diseased trees—i. e., the direction toward which the wind was blowing—show that in a general way the number of spores falling upon equal surfaces in equal intervals of time decreases as the distance from the source of spores is increased. The fact alone that in an open field at rather long distances from diseased trees ascospores were prevalent in the air to such an extent that every minute from 6 to 24 spores were settling upon a surface equal to 1 square foot (Pl. LXIII, figs. 1 and 2) indicates that at such a time many opportunities would be offered for exposed parts of undiseased trees at considerable distances from diseased ones to become infected by wind-borne ascospores.

Furthermore, these results show that the maximum distance over which ascospores might be transported by the wind was by no means obtained, and the large numbers found at the longest distances in this experiment, given in Table VIII, when a light wind prevailed, indicate that even with a relatively light wind ascospores are probably conveyed distances far greater than these.

In a consideration of the exposure plates yielding the high numbers of colonies of *Endothia parasitica* it is interesting to note the relatively large proportion of the spore content of the air formed by ascospores of this fungus at certain times (Table IX).

TABLE IX.—*Percentage of the number of spores of Endothia parasitica to the total spore content of the air, as shown by exposure-plate tests on chestnut-bark agar in 1913 at West Chester, Pa.*

Plate No.	Date.	Total number of fungous colonies.	Number of colonies of Endothia parasitica.	Percentage of colonies of Endothia parasitica to total spore content of air.	Plate No.	Date.	Total number of fungous colonies.	Number of colonies of Endothia parasitica.	Percentage of colonies of Endothia parasitica to total spore content of air.
4383	Aug. 27.....	30	16	53	5046	Sept. 20....	9	7	77
4384	...do.....	25	21	84	5047	...do.....	11	10	91
4385	...do.....	70	33	47	5048	...do.....	6	6	100
4386	...do.....	25	20	80	5049	...do.....	9	7	77
4787	Sept. 18....	20	11	55	5050	...do.....	6	3	50
5041	Sept. 20....	12	10	83	5069	Sept. 21....	30	12	40
5042	...do.....	9	7	77	5070	...do.....	50	20	40
5043	...do.....	10	4	40	5071	...do.....	70	62	88
5044	...do.....	23	22	95	5072	...do.....	28	24	85
5045	...do.....	14	11	78	5073	...do.....	21	19	90

In connection with these figures it should be borne in mind that the fungi represented are such as will grow only on chestnut-bark agar. Taking into consideration, however, the relatively large numbers of other fungi ordinarily developing in the exposure plates (Table I), it is a noteworthy fact that at certain periods when ascospore expulsion was in progress the spores of this one species should constitute from 40 to 100 per cent of the total spore content of the air.

Since these plates were exposed not long after a rain, a possible explanation suggested is that spores of other fungi were washed from the air by the rain and the supply had not yet been replenished, whereas conditions were very favorable to the abundant expulsion of ascospores of *Endothia parasitica*. It has also been suspected that certain types other than this fungus which were often found in plates exposed at such times represented other ascomycetous fungi the spores of which had just been expelled.

SUMMARY OF EXPOSURE-PLATE TESTS

In all of the exposure plates yielding colonies of *Endothia parasitica* it was determined from the time of appearance of these colonies that all originated from ascospores. Therefore we may safely state at the outset that under the conditions of the tests little or no wind dissemination of pycnospores occurred.

By comparison with ascospore-trap tests it is evident that ascospores of *Endothia parasitica* were caught in the exposure plates in numbers and at some distances from trees only during certain periods following rains when ascospore expulsion was in progress. The possible exception occurred on the morning of September 8, when no series of observations was made on the ascopore traps.

As the occurrence of ascospores in the air in considerable numbers is the prime requisite for wind dissemination and as ascospore expulsion

occurs only when the perithecia-bearing bark has been wet by rains, the following facts are presented to show that wind dissemination is directly dependent upon weather conditions causing spore expulsion.

Of the total number of 756 plates exposed during these tests 95 were exposed while ascospore expulsion was known to have been in progress, and of these, 41 yielded colonies of *Endothia parasitica*. Of the remaining 661 plates exposed at other times than those noted above, but 23 yielded colonies of *E. parasitica*, and 14 of these were exposed within 12 hours after expulsion was known to have occurred.

To bring out in a more striking manner the relation of rain to wind dissemination, it is worthy of note that out of a total of 427 ascospores of *Endothia parasitica* caught in the exposed plates 402, or 94 per cent, were caught in plates exposed while spore expulsion was known to have been in progress, and of the remaining 25 spores 3 were caught within 5 hours after the cessation of a rain (Sept. 8) and 12 more were caught within 12 hours after ascospore expulsion was known to have occurred. This leaves but 10 out of 427 spores, or 2.3 per cent, seeming to be stray ascospores bearing no relation to a rain.

As to the origin of the 22 colonies of *Endothia parasitica* appearing in plates exposed when spore expulsion was known not to be in progress (see Table II), the following points are cited to prove that they originated from stray ascospores which, after expulsion, lodged on near-by or, perhaps, distant trunks, limbs, or leaves and were subsequently loosened by the mechanical action of some agency.

1. All but one of the 21 plates containing these colonies yielded only a single colony of *Endothia parasitica* each.

2. In one colony a fragment of bark was visible at its center.

3. All except one of these spores were caught at stations more or less overhung by branches of diseased trees, and all except three were caught on stumps surrounded by sprouts.

4. Only 1 out of 192 plates exposed at unsheltered stations when expulsion was not in progress yielded a colony of *Endothia parasitica*.

If these had been stray spores that were still floating in the air since expulsion, they would have fallen just as frequently into plates exposed out in the open at unsheltered stations. During a period of ascospore expulsion following a rain it seems probable that the spores would not all be swept away by air currents but that some few would find lodgment upon near-by leaves and branches. Such lodgment is especially likely to take place if there is no noticeable wind when expulsion is in progress. Thus, it seems quite probable that the colonies obtained when perithecia were not active originated from spores dislodged from either healthy or diseased parts of trees more or less overhanging the plates.

Unless attached to a bark fragment, the path of these spores in falling would not necessarily approach the vertical, and such spores might be transported by the wind just as readily as though they were freshly expelled. This explains, perhaps, why one spore was caught in plate No. 5037, exposed 27 feet from the nearest chestnut tree. The probable reason, then, why, with this exception, such stray spores were caught only under trees is that the rareness of their occurrence in the air prevented their detection elsewhere than in very close proximity to their place of temporary lodgment, since with the exposure-plate method the chance of detecting these spores decreases very rapidly as the distance from their source is increased.

Obviously no exposures could be made during a rain, but ascospore-trap examinations have shown that abundant spore expulsion may occur during the actual fall of the rain. It is evident, however, that at such a time wind dissemination would be reduced to a minimum, because the spores upon expulsion would soon be washed to the ground or to near-by bark or foliage.

Therefore, under the conditions of our tests it can be said that, with the exception of the few stray ascospores loosened from temporary lodgment, wind dissemination of *Endothia parasitica* occurs only during certain periods after rains, when ascospore expulsion is in progress.

ASCOSPORE-TRAP TESTS

In order to detect ascospore expulsion whenever it occurred, use was made of what we have termed "ascospore traps." An ascospore trap consisted of a glass object slide held in place over perithecial pustules on the bark of a diseased tree by means of a wooden bracket either above or below the slide (Pl. LXIV, figs. 1 and 2). The slide was wedged firmly into a slot in the bracket so as to be suspended about one-eighth of an inch or less from the papillæ underneath. These traps were placed on lesions of various ages on trees more or less scattered throughout the experimental plot (fig. 1).

As the ascospores of *Endothia parasitica* are expelled they adhere to the glass, and the spores expelled from each ostiole usually form a definite "spot," so that the number of spots on the slide represents the number of perithecia in the area underneath which have expelled spores.

During the progress of the work on wind dissemination, it was found possible by means of these traps not only to detect the occurrence of ascospore expulsion but to determine even with some degree of accuracy the exact duration of perithecial activity.

As has been brought out in the discussion of the exposure-plate tests, the occurrence of ascospores in the air in numbers is directly dependent upon the continuation of their expulsion after a rain has ceased. The duration of expulsion becomes, therefore, an essential factor in determining the period during which wind dissemination may occur.

In making this determination the method of procedure was as follows: Out of the total number of 69 ascospore traps usually about 20 were selected, representing areas of vigorous perithecia where previous experience indicated that abundant expulsion was most likely to occur. The slides from these traps were collected as soon as possible after the rain and were replaced with clean slides. Then, after a convenient interval, this second set of slides was collected and replaced with clean ones. This operation was repeated at intervals of several minutes to several hours until none of the slides bore spots of expelled ascospores.

Several series of trap collections were usually made after each rain, and a subsequent examination of each slide revealed whether or not any expulsion had occurred under that trap in the period during which that particular slide had been in place on the tree. Although usually visible to the unaided eye, an examination with a hand lens was often necessary to detect very faint or very diffuse spots of ascospores on the slides.

The detailed results for September 20 are given to show the behavior of individual traps (Table X). The results given in the summary for the other dates were obtained in a similar manner and the individual records will therefore be omitted.

TABLE X.—*Record of ascospore-trap collections on September 20, 1913, at West Chester, Pa.*[a]

Time from cessation of rain to—		Number of perithecia expelling ascospores between times stated.																	
Replacing of slides.	Collection of slides.	Trap No. 59.	Trap No. 58.	Trap No. 60.	Trap No. 62.	Trap No. 63.	Trap No. 64.	Trap No. 65.	Trap No. 68.	Trap No. 69.	Trap No. 74.	Trap No. 75.	Trap No. 72.	Trap No. 14.	Trap No. 15.	Trap No. 39.	Trap No. 40.	Trap No. 45.	Trap No. 4.	Trap No. 50.
.....	2^h48^m to 3^h10^m.....	44	22	118	73	79	53	40+	14	238+	82	77	2	7	13	8	5	0	0	0
2^h48^m to 3^h10^m.....	4^h5^m to 4^h27^m.....	23	7	67	66	43	5	23	0	101	53	34	1	3	2	1	4	0	0	0
4^h5^m to 4^h27^m.....	5^h23^m to 5^h41^m.....	6	4	19	17	15	1	9	0	22	30	4	0	2	0	1	0	0	0	0
5^h23^m to 5^h41^m.....	6^h12^m to 6^h33^m.....	0	1	0	8	1	1	1	0	11	4	0	0	1	0	0	0	0	0	0
6^h12^m to 6^h33^m.....	6^h53^m to 7^h11^m.....	0	0	0	5	0	0	0	..	4	0	..	..	1	0	..	..	0	0	0
6^h53^m to 7^h11^m.....	7^h35^m to 7^h47^m.....	..	..	....	3	0	..	....	..	2	..	..	..	0	0	..	..	0	0	..
7^h35^m to 7^h47^m.....	10^h34^m to 11^h31^m.....	0	11	0	0	0	0	0	0	0	0	0	..	0	0	1	0	0	0	0
10^h34^m to 11^h31^m.....	13^h14^m to 13^h38^m.....	..	9	....	..	..	..	....	..	0	..	..	..	0	0	..	..	0	0	..
13^h14^m.....	13^h49^m.....	..	2	....	..	..	..	....	..	.....	..	..	..	..	..	..	..	..	..	..
13^h49^m.....	14^h40^m.....	..	0	....	..	..	..	....	..	.....	..	..	..	..	..	..	..	..	..	..

[a] Rainfall, night previous, 0.09 inch. Time of cessation, between 3.25 and 3.55 a. m.

TABLE XI.—*Summary of records of ascospore-trap collections in 1913 at West Chester, Pa.*

Date.	Rainfall.	Number of traps examined.	Time from cessation of rain to—		Number of perithecia expelling ascospores between times stated.
			Replacing of slides.	Collection of slides.	
	Inches.				
		19	.....	0^m to 18^m.....	0
Aug. 27....	0.12	19	0^m to 18^m.....	1^h22^m to 1^h27^m.....	1
		14	.....	0^m to 15^m.....	1
Do....	.055	14	0^m to 15^m.....	45^m to 1^h.....	360
		14	45^m to 1^h.....	14^h (Aug. 28).....	227
		19	.....	2^h6^m to 2^h37^m.....	1,047+
		19	2^h6^m to 2^h37^m.....	3^h19^m to 3^h54^m.....	53
Sept. 18....	.26	19	3^h19^m to 3^h54^m.....	4^h20^m to 4^h38^m.....	9
		19	4^h20^m to 4^h38^m.....	5^h47^m to 7^h19^m.....	35
		9	5^h47^m to 6^h37^m.....	12^h25^m to 13^h10^m.....	1
		8	.....	1^h48^m to 2^h4^m.....	236
		10	.....	3^h35^m to 5^h30^m.....	219
Sept. 19....	.68	8	1^h48^m to 2^h4^m.....	3^h35^m to 5^h30^m.....	15
		17	3^h35^m to 5^h30^m.....	12^h10^m to 13^h.....	46
		19	.....	2^h48^m to 3^h10^m.....	875+
		19	2^h48^m to 3^h10^m.....	4^h5^m to 4^h27^m.....	433
		19	4^h5^m to 4^h27^m.....	5^h23^m to 5^h41^m.....	130
		19	5^h23^m to 5^h41^m.....	6^h12^m to 6^h33^m.....	28
Sept. 20....	.09	14	6^h12^m to 6^h33^m.....	6^h53^m to 7^h11^m.....	10
		7	6^h53^m to 7^h11^m.....	7^h35^m to 7^h47^m.....	5
		18	7^h35^m to 7^h47^m.....	10^h34^m to 11^h31^m.....	12
		6	10^h34^m to 11^h31^m.....	13^h14^m to 13^h38^m.....	9
		1	13^h14^m.....	13^h49^m.....	2
		21	.....	1^h40^m to 2^h.....	2,494+
		21	1^h40^m to 2^h.....	3^h6^m to 3^h22^m.....	211
Sept. 21....	.43	19	3^h6^m to 3^h22^m.....	5^h28^m to 5^h36^m.....	0
		11	.....	33^m to 41^m.....	1,199+
		11	33^m to 41^m.....	53^m to 1^h1^m.....	14
Do....	.10	6	53^m to 1^h.....	1^h31^m.....	0
		21	.....	5^h10^m to 5^h30^m.....	577+
		21	5^h10^m to 5^h30^m.....	6^h31^m to 6^h51^m.....	27
		21	6^h31^m to 6^h51^m.....	8^h6^m to 8^h25^m.....	12
Sept. 22....	.73	21	8^h6^m to 8^h25^m.....	9^h35^m to 11^h11^m.....	3
		2	10^h39^m to 11^h2^m.....	14^h12^m to 14^h16^m.....	6
		10	.....	1^h3^m to 1^h11^m.....	618
		3	1^h3^m to 1^h11^m.....	1^h16^m to 1^h21^m.....	82
Oct. 20....	.85	[a] 4	.....	12^m to 27^m.....	245
		3	12^m to 27^m.....	22^m to 31^m.....	303
		4	20^m to 31^m.....	37^m to 43^m.....	387
		4	37^m to 43^m.....	45^m to 54^m.....	379
		4	45^m to 54^m.....	57^m to 1^h9^m.....	390
Do....	.01	4	57^m to 1^h9^m.....	1^h12^m to 1^h26^m.....	262
		4	1^h12^m to 1^h26^m.....	1^h28^m to 1^h46^m.....	152
		4	1^h28^m to 1^h46^m.....	1^h55^m to 2^h9^m.....	37
		4	1^h50^m to 2^h9^m.....	5^h21^m to 5^h54^m.....	2

[a] This summary includes the records of traps Nos. 58, 59, 72, and 74 only.

The data secured relative to the duration of ascospore expulsion after certain rains are given in a summarized form in Table XI. As will be seen, all of these, except the results obtained on October 20, bear reference to rains occurring during the progress of exposure-plate tests. Although the exact time of cessation of rain is a very important point, in the cases of September 18, 19, 20, and 22 it could not be more accurately determined, because the rains all ceased in the night. A comparison of the figures presented in these tables with the results obtained in the exposure-plate tests will show a close interrelation.

On October 20 another opportunity was offered to obtain data relative to duration of spore expulsion. By selecting only a few traps and changing the slides at much shorter intervals more in detail was learned in regard to the activity of the perithecia (Table XI).

Since the point had been often suggested that these ascospore-trap tests might not yield results typical of natural conditions because of the protection from drying afforded by the glass suspended over the bark and that because of this spore expulsion was greatly prolonged, occasion was taken on October 20 to determine the validity of this contention.

About two hours after the cessation of rain, when expulsion had apparently ceased under most of the ascospore traps that were being tested (Table XI), a number of clean slides were placed at random over other areas of perithecia-bearing bark which appeared still to be damp, to determine whether or not perithecia on bark unprotected by glass slides were expelling spores at this time. Owing to the high south wind and occasional sunshine, such promising areas of bark were found only on the north side of trunks, either where loosened bark about a bad lesion had become soaked or in locations more or less protected by sprouts or by stumps from the drying action of the wind. The slides were held in place by a cord tied around the trunk, and care was taken to prevent contact with the papillæ. The results obtained from these 22 test traps are given in Table XII.

TABLE XII.—*Record of test traps on October 20, 1913, at West Chester, Pa.*[a]

Trap No.	Time of placing slide.	Time of collection.	Results of examination.
1	11.20 a. m.	1.30 to 2.00 p. m.	0.
2	11.25 a. m.	1.30 to 2.00 p. m.	1 faint spot.
3	11.27 a. m.	1.30 to 2.00 p. m.	0.
4	11.27 a. m.	1.30 to 2.00 p. m.	0.
5	11.32 a. m.	1.30 to 2.00 p. m.	0.
6	11.35 a. m.	1.30 to 2.00 p. m.	0.
7	11.35 a. m.	1.30 to 2.00 p. m.	1 light spot.
8	11.39 a. m.	1.30 to 2.00 p. m.	0.
10	11.34 a. m.	1.30 to 2.00 p. m.	0.
11	11.47 a. m.	1.30 to 2.00 p. m.	0.
12	11.47 a. m.	1.30 to 2.00 p. m.	0.
14	11.55 a. m.	1.30 to 2.00 p. m.	0.
15	12.00 to 12.04 p. m.	1.30 to 2.00 p. m.	1 faint spot
16	12.00 to 12.04 p. m.	1.30 to 2.00 p. m.	0.
17	12.00 to 12.04 p. m.	1.30 to 2.00 p. m.	0.
18	12.00 to 12.04 p. m.	1.30 to 2.00 p. m.	0.
19	12.00 to 12.04 p. m.	1.30 to 2.00 p. m.	0.
20	12.00 to 12.04 p. m.	1.30 to 2.00 p. m.	3 spots; 1 rather heavy.
21	12.00 to 12.04 p. m.	1.30 to 2.00 p. m.	0.
22	12.17 p. m.	1.30 to 2.00 p. m.	1 light spot.

[a] Rainfall, 0.86 inch. Time of cessation, 9.09 a. m. Wind, high SSW.

SUMMARY OF ASCOSPORE-TRAP TESTS

From the standpoint of wind dissemination, the all-important feature proved beyond doubt by these tests is that in every case where ascospore expulsion occurred at all it continued for a time after the cessation of the rain, thus insuring a supply of spores in the air.

A glance over these results shows that in a general way the volume of ascospore expulsion, as measured by the character and number of spots on the slides, is greatest during or shortly after the rain and decreases more or less uniformly as the bark dries. On August 27 the rains were of the thunderstorm type, being of very short duration, and consequently the perithecia had hardly been wet for a sufficient length of time when the rain ceased. The greatest volume of expulsion occurred, therefore, a little later, evidently between 15 minutes and 1 hour after the rain had ceased.

With the exception of the rain in the afternoon of September 21, the tests of September 18, 19, 20, 21, and 22 are rather unsatisfactory, since no records could be obtained until some time after the rain had ceased. The summary for these dates (Table XI) shows that the maximum volume of spore expulsion had occurred before the first collections were made, and whether the climax occurred during the rain or shortly afterwards can not be stated. In the case of the afternoon rain of September 21 very evidently the maximum volume of spore expulsion took place before 33 minutes had elapsed after the cessation of the rain.

On October 20, after the bark had been thoroughly saturated by a rain in the night, the greatest volume of expulsion occurred within one hour after the rain. Two hours and nine minutes later a light rain of 58 minutes' duration began, and the results secured after this shower (Table XI) show that in three traps tested the greatest volume of expulsion occurred, not during the rain, but after 22 to 43 minutes had elapsed since its cessation.

As to the rate of subsidence of ascospore expulsion after the rains, Table XI shows a marked contrast between the results obtained on different dates. This has been mentioned in the discussion of the exposure plates and the relation of the subsidence of ascospore expulsion to weather conditions. In the cases of September 18, 19, 20, and 22 the duration of expulsion is seen to have been prolonged after the rains, and in all cases except September 19 the data show that the rate of subsidence was very gradual. Except for the last three hours of the duration of expulsion on September 22, fog or cloudiness and low wind prevailed, and the weather conditions were not favorable to rapid drying of the bark.

After the rains of September 21 and October 20 the rate of subsidence of ascospore expulsion was relatively abrupt and rapid, and its duration was comparatively short, especially after the second rain on September 21. Here, again, the relation of duration of expulsion to rapidity of drying of the bark is shown, since the rains on these dates were followed by brisk winds, and, except for the second rain of September 21, by rapid clearing and sunshine. Such weather conditions were, of course, very conducive to the rapid drying of the bark.

The maximum duration of ascospore expulsion as determined by these tests after each of these rains is shown in Table XIII. In considering these data the weather conditions just described should be borne in mind.

TABLE XIII.—*Maximum duration of ascospore expulsion after the cessation of rain, as determined by the examination of slides in ascospore traps at West Chester, Pa., in 1913*

Date.	Rainfall.	Maximum duration of spore expulsion after rain.	Date.	Rainfall.	Maximum duration of spore expulsion after rain.
	Inches.	*H. m.*		*Inches.*	*H. m.*
Aug. 27	0.175	45	Sept. 21	0.43	1 58
Sept. 18	.26	6 15	21	.10	40
19	.68	5 27	22	.73	11 2
20	.09	13 14	Oct. 20	.86	3 8

It should be mentioned in this connection that the figures given in the Table XIII were secured in all cases, except that of October 20, from bark that had been protected continuously by the trap slide from the drying action of the wind, and it is possible that under such conditions the duration of expulsion may be slightly prolonged. But the data relative to the maximum duration of expulsion on October 20 were secured from bark previously unprotected by slides, since seven perithecia in five exposed areas were found to be expelling spores after expulsion had ceased in all but one area protected by the ascospore traps (Table XII). These tests prove beyond doubt that under natural conditions certain exposed areas of diseased bark do remain wet enough to cause spore expulsion fully as long as the particular areas protected by the ascospore traps. Of course, such areas would usually be in locations more or less protected from the wind or sun; but, nevertheless, they would continue to act as a source of spores for wind dissemination as long as any expulsion was in progress.

The direct bearing of the results of these ascospore-trap tests upon the results obtained in the exposure plates has been brought out in the discussion of the latter topic.

ASPIRATOR TESTS

It has already been brought out in the historical introduction that previous analyses of air by the aspirator method under natural conditions in the field during dry weather failed to show the presence of spores of the chestnut-blight fungus (2). Positive results were obtained, however, under artificial conditions in the field, and it seems probable that failure to detect spores under natural conditions was due to the fact that most of the analyses were made during dry weather. If positive results were obtained following periods of rain, that fact was not brought out in the discussion (2). In order to obtain definite information on this point, the aspirator tests reported in the following pages were made so as to include the filtration of air immediately following periods of rain, as well as during the intervening dry weather.

METHOD OF MAKING THE ANALYSIS

The apparatus used in this series of tests consisted of a 4-liter aspirator bottle set on a level stump near the center of the field (fig. 1). The nearest trees were 15 feet north, 19 feet east, and 33 feet west, and the

nearest lesion was on a branch 13 feet to the north. The standard sugar-tube method of making a quantitative bacteriological analysis of air was employed. The bottle was refilled with 4 liters of water at intervals of 20 or 30 minutes, thus making the aspiration practically continuous. One sugar tube was generally used each day, and the quantity of air drawn through each tube averaged 58 liters, with a maximum of 96 liters. The medium employed was a 3 per cent dextrose agar, with a reaction of +10. Ten plates were poured for each test and were incubated and the colonies counted in the same way as those in the experiments with the water spore traps (p. 520).

TABLE XIV.—*Summary of results of aspirator tests in 1913 at West Chester, Pa.*

Sugar tube No.	Date of aspiration.	Rainfall.[a]	Quantity of air represented.	Number of bacteria and yeasts per liter.	Total number of fungi per liter.	Number of spores of Endothia parasitica per liter.	Number of species of fungi.
		Inches.	*Liters.*				
1	Aug. 19	0.40	12	26.42	4.16	0	4
2	Aug. 20	0	28	3.21	4.28	0	9
3	Aug. 21	0	36	.83	3.05	0	9
4	Aug. 22	0	48	2.29	5.21	0	7
5	Aug. 23	.25	68	1.47	17.65	0	3
6	Aug. 24	0	56	2.86	11.07	0	5
7	Aug. 25	0	56	3.96	3.96	.35	7
8	Aug. 26	0	56	4.64	8.93	0	10
9	Aug. 27	.175	47	2.12	8.3	0	11
10	do	.....	12	3.75	5.0	.42	9
11	Aug. 28	0	68	1.35	4.04	0	12
12	Aug. 29	.....	40	2.5	5.875	0	9
13	Aug. 30	1.10	60	1.25	3.91	0	7
14	Aug. 31	0	56	1.16	7.67	0	10
15	Sept. 1	0	92	1.25	38.37	0	11
16	Sept. 2	0	72	1.18	1.87	0	8
17	Sept. 3	0	72	1.32	7.91	0	12
18	Sept. 4	0	56	1.78	36.60	0	11
19	Sept. 5	0	68	1.69	20.00	0	11
20	do	0	4	16.78	6.25	0	7
21	Sept. 6	0	76	1.12	8.09	0	12
22	Sept. 7	.....	64	4.61	3.90	0	13
23	Sept. 7 and 8	.37	12	6.25	8.33	0	10
24	Sept. 8	.....	76	.39	4.86	0	10
25	Sept. 9	0	60	.16	1.41	0	5
26	Sept. 10	0	76	2.17	6.90	0	10
27	Sept. 11	0	72	.....	1.94	0	7
28	Sept. 12	.....	80	1.50	2.58	0	.....
29	Sept. 13	.095	68	.59	1.91	0	9
30	Sept. 14	0	72	.14	1.11	0	6
31	Sept. 15	0	72	.62	1.18	0	10
32	Sept. 16	0	72	.41	1.25	0	6
33	Sept. 17	.....	80	1.00	1.12	0	8
34	Sept. 18	.26	52	7.59	14.23	.192	8
35	do	.....	64	.15	5.94	0	14
36	Sept. 19	.68	96	1.04	4.27	0	10
37	Sept. 20	.09	76	.59	1.31	0	9
38	Sept. 21	.43	40	1.50	4.50	.125	8
39	do	.10	28	1.52	6.25	.089	10
40	Sept. 22	.73	52	2.30	1.82	0	6
41	Sept. 23	0	80	.875	1.31	0	.....
	Average	.....	57.9	2.91	7.03	.....	8.8

[a] All rains occurred during the night previous to the date of aspiration, except on Aug. 27.

DISCUSSION OF RESULTS OF ASPIRATOR TESTS

The results obtained from these tests are presented in Table XIV. The average number of bacteria per liter of air was 2.91, while the number of fungi per liter averaged 7.03. The number of fungus species represented in the cultures ranged from 3 to 14.

In only five instances did any colonies of the chestnut blight fungus appear in culture, and the number of spores per liter was never large. It is not impossible that the small numbers of spores of *Endothia parasitica* obtained may be due to the effect of sunlight, for in those instances where the rains were followed by fair weather the aspirator was exposed to the direct rays of the sun for a part of the day. This may also be the explanation of the fact that no spores of *E. parasitica* were obtained after some of the rains when ascospore-trap collections made it certain that expulsion was taking place, notably those of September 7 and 8 and September 21 and 22 (Table XV). Unfortunately there are no published investigations which give any information on the effect of sunlight on ascospores of the chestnut-blight fungus.

TABLE XV.—*Relation of aspiration tests to rainfall in 1913 at West Chester, Pa.*

Date of rain.	Rainfall.	Date of aspiration.	Quantity of air tested.	Number of spores of *Endothia parasitica* to 10 liters of air	Results with exposure plates.
	Inches.		*Liters.*		
Aug. 27	0.175	Aug. 27	12	4.2	+
29–30	1.10	30	60	0	−
Sept. 7–8	.37	Sept. 7–8	12	0	+
12–13	.095	13	68	0	−
17–18	.26	18	52	1.92	+
18–19	.68	19	96	0	+
19–20	.09	20	76	0	+
20–21	.43	21	40	1.25	+
21	.10	21	28	.89	+
21–22	.73	22	52	0	+

The chief explanation of the small number of spores of *Endothia parasitica* to the liter is to be found in the small amount of air drawn through each tube. While this averaged 38 liters for those tubes yielding positive results, only a few liters were drawn through the tube in the several hours during which copious expulsion of ascospores took place. The figures given in the tables are therefore smaller than the actual number of spores per liter during the period of copious expulsion. In view of these facts, Tables XIV and XV do not represent the true number of ascospores present in the air during the time of their actual prevalence, since the period of aspiration included many hours when they were not prevalent, as shown by the exposure-plate tests.

The rate of development of the colonies of the chestnut-blight fungus showed that they all originated from ascospores and none from pycnospores (5).

The spores obtained from sugar tube No. 7 two days after a rain may have been stray spores similar to those obtained in several exposure plates.

The aspirator tests do not appear to have given as reliable results as the exposure-plate method, since it may be noted from Table XV that negative results were obtained on certain days when the exposure plates showed that ascospores were prevalent. The importance, however, of the aspirator tests lies in the fact that ascospores were obtained under perfectly natural conditions in the field at a distance of 13 feet from the nearest lesion and that they were obtained at times when ascospore expulsion was taking place.

WATER SPORE-TRAP TESTS

The use of water spore traps for testing the transport of spores of the chestnut-blight fungus by the wind was the outcome of our attempts to use the method of Burrill and Barrett (5) in their study of the wind dissemination of *Diplodia zeae*. First, substituting a funnel for the glass plates employed by the writers just cited, an attempt was made to find some mixture which could be applied to the inner surface of the funnel and which would fulfill the necessary requirements, as follows:

1. The mixture must contain no substances toxic to spores of the chestnut-blight fungus.
2. It must spread readily and adhere to a glass surface.
3. It must be sticky, so as to retain the spores which lodge upon the surface, which is coated with it.
4. It must retain its sticky character at least 24 hours under field conditions.
5. It must be readily soluble in water.

Glycerin of various percentages was tried alone, as well as in combination with various quantities of gum arabic or gelatin, but in all cases the mixtures either dried too soon or did not spread well on a glass surface.

The fact that pycnospores do not germinate in water (4) suggested the substitution of dishes of sterile water for the funnels. The first idea was that analyses of the water from these dishes exposed in the field under natural conditions could be made at intervals of some days and would reveal the presence of pycnospores if they had been carried by the wind. Experience in the field, however, proved that the method was also well adapted to the study of ascospore dissemination.

DESCRIPTION OF THE WATER SPORE TRAPS

A water spore trap consisted of a crystallizing dish 5 cm. deep and 10 to 12 cm. in diameter, into which sterile water was introduced. The dishes were wrapped in paper and sterilized in the laboratory for transport to the field. Each dish was supported about 2 feet above the ground by a tripod of three small stakes driven into the ground. Ten-penny nails were driven into the ends of the stakes, whose ends were converged to make a support for the dish. The nails were held in proper position by a heavy cord attached to them and encircling the dish. By this means they were so firmly secured that they were never in danger of being blown out by the wind (Pl. LXIV, fig. 3). After placing a dish in its proper field location, 100 to 150 c. c. of sterile water were introduced. Water for this purpose was kept in stock in small Erlenmeyer flasks.

The dishes of water were exposed in the field in various selected locations and analyses made at certain intervals (fig. 3; also Pl. LXV, figs. 1 and 2).

METHOD OF MAKING A TEST

At the end of an exposure period the contents of each dish were emptied into sterile flasks provided for the purpose and transported to the laboratory at the University of Pennsylvania, where the work of making an analysis was completed. They were then replaced with other sterile dishes and sterile water introduced as before.

For each water spore trap 15 to 20 plate cultures were employed, and these were made by introducing 0.1 to 0.5 c. c. of the water by means of a graduated 1 c. c. pipette into each Petri dish. In this way only 4 to 5 c. c. of the total water returned to the laboratory were used in each

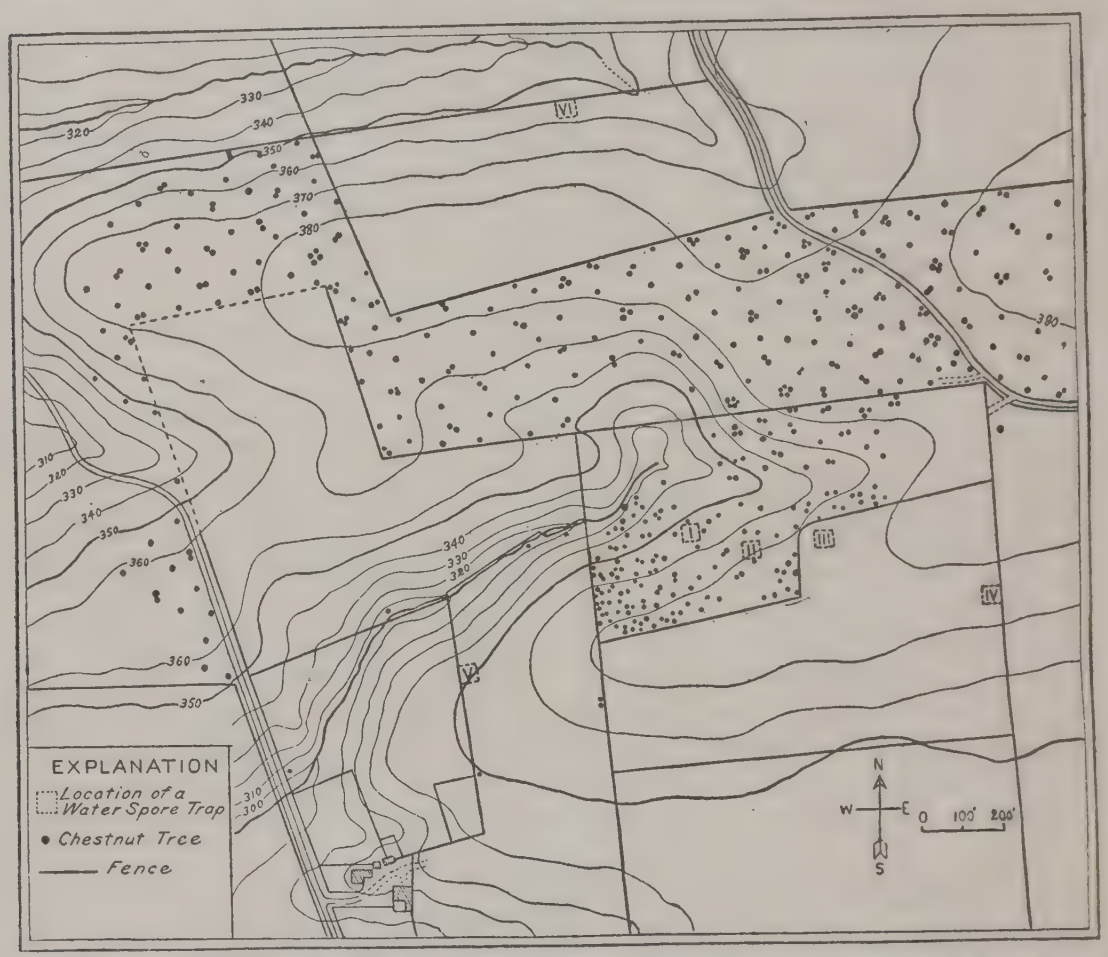

FIG. 3.—Map showing the location of water spore-trap stations Nos. I to VI. Stations I and II are in the chestnut coppice, the detailed composition of which is shown in figure 1; Stations III to V are at various distances from the same coppice; Station VI is to the north of a mixed chestnut and oak woodland.

test, but special pains were taken to secure a uniform suspension before the removal of the quantities used. Chestnut-bark agar was used for all of these analyses (see p. 496 for formula) since experience had proved that it was a poor medium for the growth of bacteria, which were always present in some quantity. In fact, the medium is so unfavorable for the development of ordinary bacteria that in most cases the colonies remained as minute specks during the period the plates were under observation and with proper dilution offered no hindrance to the development of colonies of *Endothia parasitica* and other fungi. All cultures were incubated as nearly as possible at 25° C., and the colonies of fungi suspected of being the chestnut-blight fungus were marked at the end of three days. The count was completed on the fifth day, and any uncertain colonies

were transferred to 3 per cent dextrose agar for further study. In general, it may be said that transfers were not necessary, for the colonies of *E. parasitica* are very characteristic on chestnut-bark agar at the end of five days, if they have had sufficient room in which to develop. It was only in the case of plates badly crowded with other fungi that such transfers were necessary.

RESULTS AND DISCUSSION OF TESTS

The water spore traps were exposed in or near the same plot of badly diseased chestnut trees at West Chester, Pa., which was employed for the exposure plates previously reported. Six different stations were selected for the location of water spore traps at distances varying from 15 to 389 feet from the nearest blight lesions, although Station V was 404 feet from the nearest probable source of spores. More detailed information in regard to these stations is given in Table XVI.

TABLE XVI.—*Relation of water spore-trap stations to diseased chestnut trees in 1913 at West Chester, Pa.*

Trap station No.	Distance and direction from station to nearest lesions.	Position of station with reference to diseased chestnut trees.
I.....	25 feet west and east.....	Surrounded by 15- to 18-year-old coppice.
II.....	15 feet southwest; 19 feet north.	Do.
III.....	50 feet north; 50 feet west..	In cornfield 50 feet from coppice.
IV.....	383 feet northwest.....	Across cornfield from coppice.
	398 feet north.....	Across open field from a single tall tree.
V.....	237 feet northwest.....	Across pasture from a single tall tree.
	265 feet south.....	Do.
	317 feet northeast.....	Across cornfield from a single tall tree.
	404 feet east.....	Across cornfield from coppice.
VI.....	389 feet south.....	Across hayfield from older forest.

TABLE XVII.—*Summary of the tests with water spore traps in 1913, at West Chester, Pa.*

Test No.	Trap station No.	Culture Nos.	Period of exposure.	Date of cultures.	Total number of fungous spores.	Number of spores of Endothia parasitica.
1	I	4227–4234	Aug. 30 to Sept. 4..	Sept. 5..	11, 215	0
2	II	4235–4242	.....do.....	...do.....	13, 868	0
3	III	4243–4250	Aug. 31 to Sept. 4..	...do.....	23, 239	0
4	III	4821–4835	Sept. 4 to Sept. 8...	Sept. 10..	1, 236	0
5	I	4906–4920	Sept. 4 to Sept. 13..	Sept. 16..	6, 375 (?)	0
6	I	4951–4966	Sept. 13 to Sept. 18.	...do.....	10, 666	1, 749
7	II	4967–4976	.....do.....	...do.....	10, 583	3, 507
8	III	4983–4992	.....do.....	...do.....	7, 886	2, 113
9	I	5303–5312	Oct. 13 to Oct. 20...	Oct. 21...	.....	0
10	VI	5313–5322	Oct. 20, a. m.–p. m.	...do.....	1, 666	30
11	IV	5345–5354	Oct. 19 to Oct. 22..	Oct. 23..	.....	0
12	V	5355–5364	.....do.....	...do.....	.....	0
13	I	5371–5380	Oct. 22 to Oct. 27..	Oct. 28...	22, 139	378
14	IV	5381–5390	.....do.....	...do.....	13, 148	380
15	V	5391–5400	.....do.....	...do.....	4, 591	820
16	VI	5401–5410	.....do.....	...do.....	113, 203	431
17	I	5411–5420	Oct. 27 to Nov. 10..	Nov. 11..	Numerous.	0
18	V	5421–5430	.....do.....	...do.....	Numerous.	0
19	VI	5431–5440	.....do.....	...do.....	Numerous.	0

The map (fig. 3) shows the location of the coppice growth and other chestnut trees than those used in the test, with the position of the exposure stations. The character of the diseased coppice growth is shown in Plate LXV, fig. 1, which shows a view taken from Station V. The older forest, which was the source of the ascospores for the traps exposed at Station VI, is shown in Plate LXV, fig. 2. The period from August 30 to November 11, 1913, was covered by the tests presented in Table XVII.

The time and amount of rainfall, and in some cases the wind direction, are necessary in interpreting the results. Table XVIII gives the rainfall for the time covered by the water spore-trap tests.

TABLE XVIII.—*Rainfall record for period covered by the water spore-trap tests in 1913 at West Chester, Pa.*

Date of rain.	Rainfall.	Date of rain.	Rainfall.	Date of rain.	Rainfall.
	Inches.		*Inches.*		*Inches.*
Aug. 29 and 30	1. 10	Sept. 21 and 22	0. 83	Oct. 20.....	0. 08
Sept. 7 and 8	.37	30.....	.02	24.....	1. 44
12 and 13	.095	Oct. 1.....	1. 12	25.....	
17 and 18	.26	2.....	.12	26.....	
18 and 19	.68	3.....	.06	Nov. 8 and 9..	1. 00
19 and 20	.09	11.....	.73		
20 and 21	.43	19.....	.86		

Tests Nos. 1, 2, and 3 were started in the field after the rain of August 29 and 30, late in the day, and the traps were taken to the laboratory for analysis before the next rain. Judging from the results obtained from our exposure plates, no ascospores should have been present, and our failure to get any colonies of the chestnut-blight fungus in the test cultures suggests that during that period there was no wind dissemination of either pycnospores or ascospores. There was a small amount of rain during the period that traps 4 and 5 were exposed, but the analyses were not made until two and three days later. Considering the fact that ascospores germinate at once in water, the failure to get any colonies of the *Endothia parasitica* in these tests is not surprising and again points to the absence of pycnospores. Traps 6, 7, and 8 were removed from the field a few hours after the heavy rain of September 18 and 19, and the analyses gave a large number of colonies of the chestnut-blight fungus. It appears probable that the spores were caught during the few hours following the rain, since the cultures indicated the origin of the colonies from ascopores only (5). It should be noted from Tables XVII and XVIII that traps 9 to 12 were removed from the field just following periods of rain. The wind was blowing from the infected trees toward trap 10 only, and this was the only one in the series which yielded the blight fungus. Traps 13 to 16 were removed from the field shortly after the rainy period of October 24 to 26, and all yielded positive results, trap 16, located 389 feet from the nearest chestnut tree, giving 431 spores. The length of time after the rain when the tests were made and the direction of the wind are the possible explanation for the negative results for traps 17 to 19. Unfortunately no traps were exposed during the rainy periods of October 1 to 3 and October 11.

It is probable that the figures recorded for tests Nos. 6 to 8 and 13 to 16 represent the number of spores blown into the traps during the few hours

following the rain. This appears to be substantiated by negative results obtained during dry periods and by positive results obtained with exposure plates and slide traps just following a period of rain. The results are briefly summarized in Table XIX. It is interesting to note the number of viable ascospores of the chestnut-blight fungus that must have fallen on each square inch of water surface for the time represented. This information is presented in Table XX.

TABLE XIX.—*Summary of positive results obtained from water spore traps in 1913 at West Chester, Pa.*

Station.	Number of tests represented.	Distance of station from nearest lesion.	Total number of spores of Endothia parasitica caught, as determined by cultures.
		Feet.	
I	2	25	2,136
II	1	15	3,507
III	1	50	2,113
IV	1	383	380
V	1	237	820
VI	2	389	461

TABLE XX.—*Number of ascospores of Endothia parasitica falling on each square inch of water surface at various distances in 1913 at West Chester, Pa.*

Test No.	Surface area of water trap.	Number of spores of Endothia parasitica falling on each square inch of water surface.	Distance to nearest lesion.	Test No.	Surface area of water trap.	Number of spores of Endothia parasitica falling on each square inch of water surface.	Distance to nearest lesion.
	Sq. inches.		*Feet.*		*Sq. inches.*		*Feet.*
6	12.5	139	25	14	16.5	23	383
7	12.5	280	15	15	16.5	50	237
8	12.5	169	50	16	16.5	26	389
13	16.5	23	25				

The large number of spores of *Endothia parasitica* falling on each square inch of surface for a single rainy period certainly emphasizes the fact that healthy trees in the vicinity of badly diseased ones have innumerable opportunities to become infected by wind-borne spores.

It should be mentioned in this discussion of the results obtained by the water spore traps that there are some possibilities of error. It might be claimed that the spores found in the water traps were carried by birds or insects. This, however, appears exceedingly improbable. The cultures always indicated ascospores and tests have shown that birds are carriers of pycnospores only (8). The position of the traps was such as to reduce the insect visitors to a minimum. Insects tested as carriers of the chestnut-blight fungus yielded both pycnospores and ascospores, but the former were very much more abundant (17). Besides, it was rare that any insects were found in the exposure dishes. Furthermore, spores were present in the traps only at periods following rains when other tests had indicated their prevalence.

CONCLUSIONS

(1) As a result of 756 exposure plates made in or near the badly diseased chestnut coppice at West Chester, Pa., it can be definitely stated that ascospores of *Endothia parasitica* (Murr.) And. are prevalent in the air and after expulsion are carried for varying distances from their source.

(2) As shown by the same exposure plates, the period of prevalence of ascospores varies with the conditions following the cessation of rains; when there is a rapid drying of the bark, this period is short, but when drying is retarded, this period is correspondingly extended. The tests indicate a general prevalence of ascospores within the first 5 hours following the cessation of rains, with less abundance during later hours. The longest period for our entire series was 14 hours.

(3) During periods of dry weather ascospores, although not generally prevalent, may occasionally be detected by the exposure-plate method. These are apparently stray ascospores expelled during some previous period of rain and now loosened from lodgment on some near-by objects.

(4) In and near badly diseased chestnut groves or forests the number of ascospores falling on each square foot of exposed surface following a period of rain, as indicated by exposure plates, is very large and is sufficient to offer abundant opportunity for new infections.

(5) Ascospores are forcibly expelled in large numbers from the perithecia during and after each warm rain in case the amount is sufficient to soak up the pustules. Following a dry period a rain of 0.18 to 0.25 inch has been observed to cause copious expulsion of ascospores, while rains of 0.01 to 0.10 inch, if immediately preceded by a copious rainfall, have been sufficient to cause the resumption of spore expulsion.

(6) As determined by the ascospore traps, the duration of expulsion depends on the rapidity with which the bark dries and only continues when the stromata are moist. Under natural conditions in the field the period of expulsion for eight rains varied from 45 minutes to 13 hours and 14 minutes.

(7) In some cases at least the maximum of ascospore expulsion occurs after the cessation of rain.

(8) The fact that the period of ascospore expulsion as determined by the ascospore traps coincides in general with the period during which spores were obtained by exposure plates points to these forcibly expelled spores as the ones prevalent following periods of rain. This is definitely substantiated by the development of colonies in the exposure plates from ascospores only.

(9) It is possible to determine the presence of ascospores of the chestnut-blight fungus in the air under natural conditions in the field by the standard aspirator method of bacteriological analysis. By this method positive results were obtained following four different rainy periods, but only when the period of aspiration included a period of copious ascospore expulsion.

(10) By the use of water spore traps stationed at varying distances from diseased trees it was possible to determine that ascospores are prevalent in the air and fall upon exposed surfaces in considerable numbers, the number diminishing with the distance from the source of supply.

(11) By making possible long exposures the water spore traps offered some advantages over the exposure-plate and aspirator methods. The presence of spores of the chestnut-blight fungus, however, was never shown by this method unless the period of exposure included a period of ascospore expulsion.

(12) The failure to obtain colonies of the *Endothia parasitica* from the water spore traps exposed during dry periods, as well as the fact that only ascospore colonies were indicated in the aspirator and exposure-plate tests, points to the conclusion that pycnospores are not generally prevalent in the air at any time. If present they certainly would be detected by the prolonged exposure of water spore traps.

(13) The time immediately following a rain, when the bark is still moist, would appear to be a favorable one for new infections, since the supply of moisture would offer opportunity for germination of spores. It is a noteworthy fact that it is only during this favorable period for germination that the dissemination of ascospores takes place.

(14) All of these experiments point to air and wind transport of the ascospores of the chestnut-blight fungus as one of the very important methods of dissemination and substantiate the conclusions of Rankin (15, 16) and Anderson (1, 2). It can now be said with absolute certainty that following each warm rain of any amount ascospores are carried away from diseased trees in large numbers. Since they have been obtained in large numbers at distances of 300 to 400 feet from the source of supply, the conclusion of the authors that they may be carried much greater distances is justified. During dry periods wind dissemination of ascospores does not occur at all or sinks to a very insignificant minimum.

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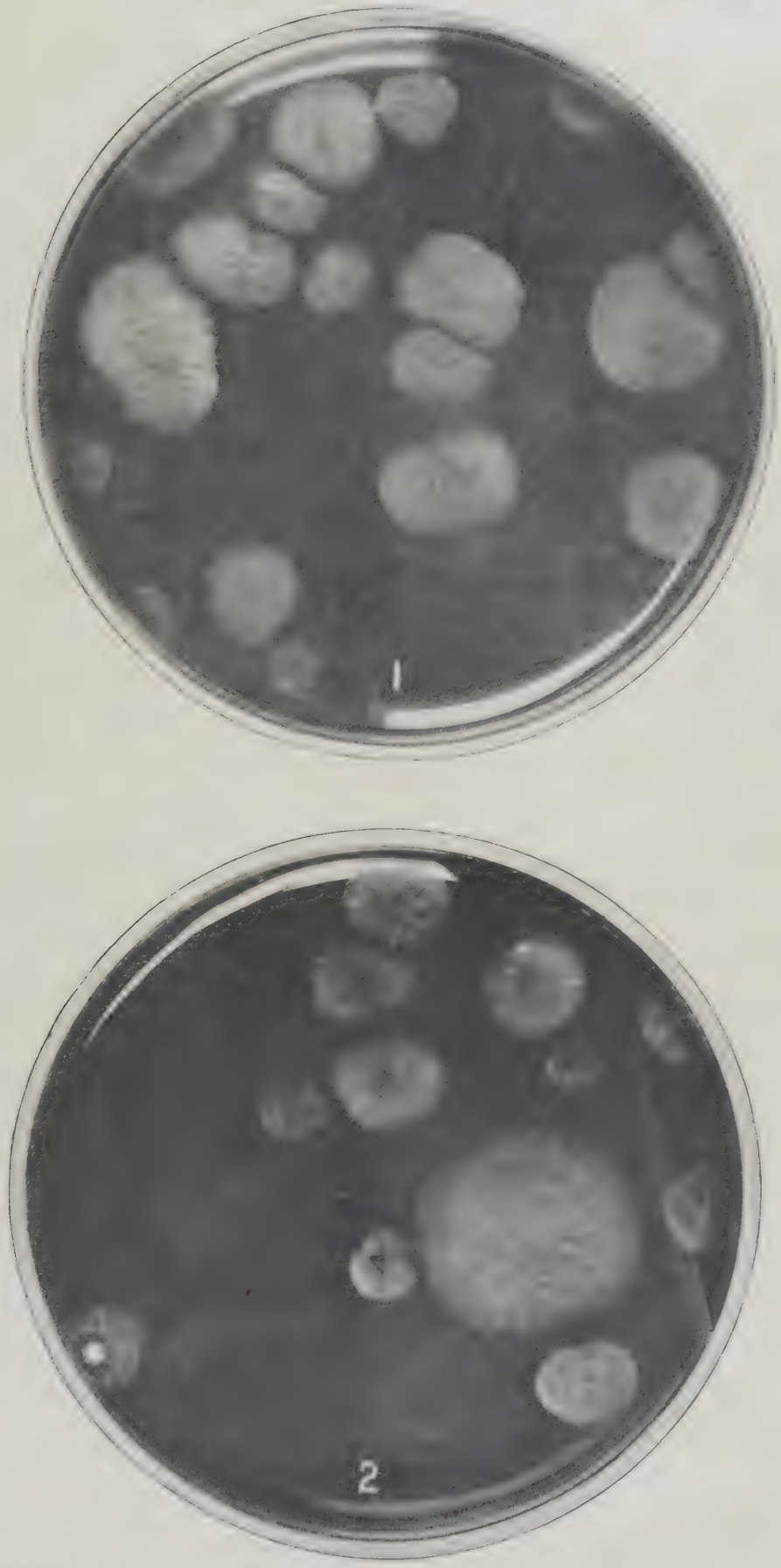
PLATE LXIII

Fig. 1.—Petri-dish culture 5044 from 12 minutes' exposure of chestnut-bark agar, made on September 20, 1913, 2 hours and 8 minutes after the cessation of a rain, at station 51, located 27 feet from the nearest lesion.

Fig. 2.—Petri-dish culture 5041 from 16 minutes' exposure of chestnut-bark agar, made on September 20, 1913, 1 hour and 55 minutes after the cessation of a rain, at station 49, located 414 feet from the source of the spores. Ten of the twelve colonies are those of *Endothia parasitica*.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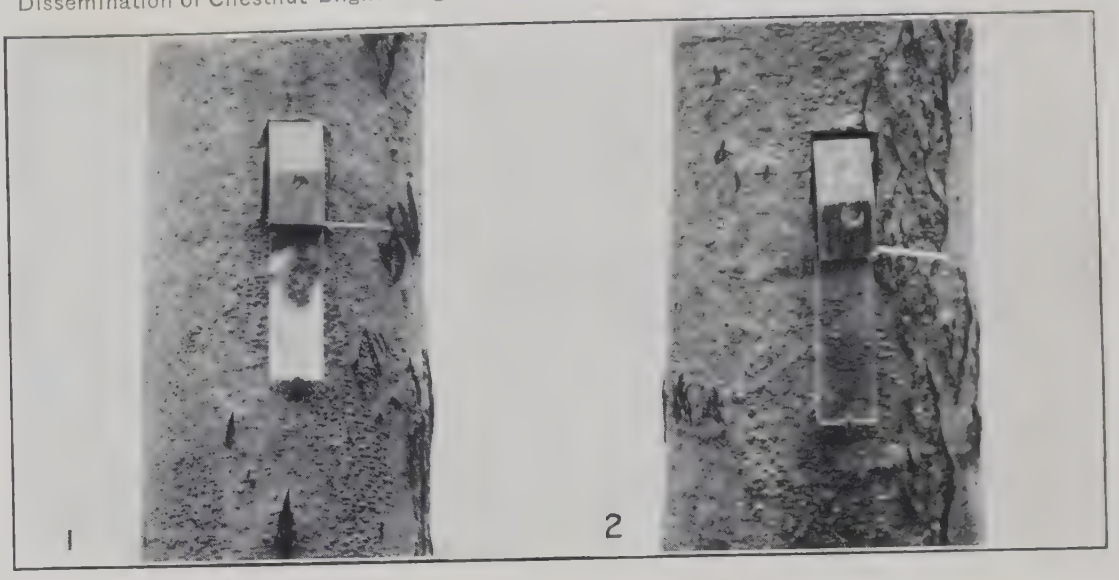
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PLATE LXIV

Fig. 1.—Ascospore trap 51. This consists of a wooden bracket which supports an object slide over perithecial pustules.

Fig. 2.—Ascospore trap 52.

Fig. 3.—Water spore trap located at Station V. The trap consists of a crystallizing dish containing sterile water and is supported on a tripod.

PLATE LXV

Fig. 1.—View looking towards the coppice growth from water spore-trap Station V. The trees in the background at the right are at the end of the plot shown in text figure 1.

Fig. 2.—View of a mixed chestnut and oak grove taken from water spore-trap Station VI. This grove was the source of the spores of the blight fungus caught at Station VI.

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